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XXIX CICLO

MENTAL STEPS:

MOTOR IMAGERY OF GAIT IN ELDERLY AND ITS ROLE IN REHABILITATION

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Abstract

La deambulazione è un comportamento altamente automatizzato e iperappreso, ma basato su programmi sensori motori complessi che coinvolgono numerosi centri nel tronco encefalico, nel cervelletto e nella corteccia. Il decadimento delle abilità di deambulazione è uno dei tratti che definiscono l'invecchiamento. L'osteoartrite per gli arti inferiori è considerato la maggior causa di disabilità e handicap nelle società occidentali industrializzati: è la causa maggiore di dolore muscolo scheletrico e a causa della grave limitazione funzionale molte attività della vita quotidiana sono compromesse. La comprensione della fisiologia del cammino a livello centrale, i cambiamenti dovuti all'invecchiamento o l'impatto di patologie periferiche su questa fisiologia è molto limitato.

In questa tesi ho descritto una serie di esperimenti con lo scopo di comprendere meglio la fenomenologia del cammino negli anziani e definire quanto questa abilità e la sua rappresentazione mentale, evocata tramite specifici compiti, potesse essere influenzata dall'osteoartrite al ginocchio.

Questa patologia è stata selezionata come modello di un deterioramento delle rappresentazioni del cammino a livello cortico/sottocorticale in assenza di chiari disturbi neurologici.

Lo scopo a lungo termine di questa ricerca è dare un solido razionale per poter testare l'efficacia di una riabilitazione basata su strategie di immaginazione motoria nel recupero post chirurgico.

Tramite gli esperimenti ho definito la neurofisiologia del cammino a livello centrale (sopraspinale) nell'immaginazione motoria e nell'immaginazione dell'imitazione, tramite l'uso della fMRI. Questo è stato fatto prima nei sani. Nel mio esperimento finale il pattern fMRI ottenuti per i soggetti sani sono stati confrontati con quelli di un gruppo di pazienti con osteoartrite del ginocchio.

I punti principali possono essere così riassunti: (1) i pazienti con osteoartrite al ginocchio sono ancora capaci di eseguire compiti di immaginazione motoria che coinvolgono la deambulazione; (2) ciononostante sembra che i pazienti non siano stati in grado di incorporare la loro limitazione motoria periferica nella simulazione mentale della deambulazione in quanto risultano più veloci dei loro controlli sani; (3) i dati fMRI per i soggetti sani mostrano che l'immaginazione motoria per il cammino dipende da un grande pattern fronto-parietale a livello corticale e maggiori attivazioni a livello cerebellare e del tronco encefalico, in aree legate al cammino, rispetto all'immaginazione dell'imitazione.(4) Infine i dati fMRI per i pazienti con osteoartrite hanno mostrato maggiori attivazioni per aree coinvolte nell'immaginazione motoria quando il compito di immaginazione era combinato con un'esplicita simulazione della deambulazione ossia la dorsi-flessione delle caviglie.

Insieme questi risultati (1) contribuiscono alla definizione dei pattern neurali coinvolti nell'immaginazione del cammino per cervelli sani, (2) dimostrano abilità qualitativamente differenti, ma ancora accessibili, nella rappresentazione della deambulazione per compiti di

immaginazione motoria sia a livello comportamentale (3) che a livello anatomo-funzionale. Il razionale di questo studio lascia ipotizzare che l'uso dell'immaginazione motoria possa facilitare il recupero della deambulazione in pazienti con osteoartrite al ginocchio nel periodo post operatorio, magari aggiungendo qualche particolare aggiuntivo, come minimo comportamento motorio (come la dorsi-flessione della caviglia). Questo mi sarà possibile discuterlo nel momento in cui avrò terminato gli specifici esperimenti per l'uso dell'immaginazione motoria in riabilitazione.

Abstract

Gait is a highly automatic behavior. Although walking is an over-experienced action, stance and locomotion are based on complex sensorimotor programs that involve several distinct and separate supraspinal centers in the brainstem, cerebellum and the cortex (l'italiano sarebbe: Gait is a highly automatic and over-experienced behavior, based on complex sensorimotor programs that involve several centers in the brainstem, cerebellum and the cortex). The decay of gait related skills is one of the defining traits of ageing. Osteoarthritis in the lower limbs is considered the single most important cause of disability and handicap in Western industrialized countries: it is the main cause of musculoskeletal pain, and daily life activities are reduced due to severe functional limitation. The comprehension of the central physiology of walking and its age-related changes or the impact of peripheral disease on this physiology is very limited.

In the present thesis I describe a series of experiments whose aim was to better understand the phenomenology of walking in elderly people and to assess to what extent this skill and its mental representation, evoked through specific motor imagery tasks, can be affected by a peripheral disorder such as knee osteoarthritis. This disease was chosen as a model of possible deterioration of cortical/subcortical representations of walking behavior in the absence of obvious neurological disorders.

The long-term goal of this research is also to test the beneficial effect of motor-imagery-based rehabilitation strategies in guiding post-surgery recovery of the patients. The present study aims at providing a strong rationale for this overarching goal.

In the same series of experiments, I characterize the central (supraspinal) neurophysiology of walking using fMRI in motor imagery or imitation through imagery. This is done in normal subjects first. In my final experiment I compare the fMRI patterns of normal subjects with those of patients with knee osteoarthritis.

The main points of my experiments can be summarized as follows: (1) patients with knee osteoarthritis are still capable of motor imagery for the walking behavior; (2) yet, they seem not to have incorporated their peripheral motor limitation in the walking simulation performed during imagery as they are comparatively faster in motor imagery than the normal controls, once the time taken to walk is subtracted; (3) the fMRI data on normal controls showed that motor imagery of walking in normal controls depends on a rich fronto-parietal pattern at the cortical level with stronger activation of cerebellar and brainstem gait specific regions for motor imagery rather than imitation through imagery task. (4) Finally patients with knee osteoarthritis displayed stronger fMRI activations in walking-specific brain regions for motor imagery, compared with normal

controls, providing that the motor imagery task was performed in combination with an explicit simulation of gait through explicit ankle dorsiflexion.

Taken together, these results (1) contribute to the definition of the normal brain patterns associated with simulated gait, (2) testify to a qualitatively different, yet still available, ability in representing a walking behavior through motor imagery in patients with knee osteoarthritis both at a behavioral and (3) at a functional anatomical level. With some additional care, like the combination of the execution of a minimal peripheral motor behavior (the ankle dorsiflexion), the present data provide a rationale to test the hypothesis that motor imagery may prove of some use in boosting motor recovery of walking in patients with knee osteoarthritis after surgery. This is something that I should be able to discuss in person when specific experiments on motor imagery in motor rehabilitation will be completed.

Summary

In my thesis, I present a new attempt to better understand the functional anatomical foundation of gait AO, MI, and action execution (AE) as measured by gait-related tasks performed in the fMRI context. The same fMRI protocol was used to compare a population of healthy subjects and a group of patients, candidates to total knee arthroplasty (TKA), recruited the day before surgery, to observe the neurofunctional reduction due to osteoarthritis. Moreover, I will present the comparison between behavioral imagined and executed tasks, respectively involving the lower and the upper limbs, to test different levels of complexity between MI and AE based on the effectors used.

After a theoretical introduction in **Chapter 1**, I will describe the differences in the characteristics of a group of candidates for TKA (**Chapter 2**) tested in a series of MI tasks involving the movements of both the upper and lower limbs divided in two levels of complexity, and compared to a control group. Gait was used as complex task for the lower limbs. The goal of the experiment was to find whether a pure peripheral functional limitation, such as the one reported by our patients, would influence both their AE and MI performances for the gait-related task. Patients with knee osteoarthritis are still capable of motor imagery for the walking behaviour (they show highly significant correlations between the time taken for real walk and time taken to imagine the same walk); yet, they seem not to have incorporated their peripheral motor limitation in the walking simulation performed during imagery as they are comparatively faster in motor imagery than the normal controls, once the time taken to walk is subtracted out.

In **Chapter 3** I will describe the neurofunctional activations obtained for a group of 21 healthy subjects, as measured by two fMRI experiments that required to imagine walking while watching moving scenarios or to imagine imitating the locomotion of different actors shown in the same scenarios. Both tests were repeated twice, with and without the association of ankle dorsiflexion. Results showed that the MI task recruited both cortical and subcortical networks consistent with locomotion, also including the so-called mesenchephalic locomotor region (MLR), the region controlling the pace and rhythm of gait. The network identified by the MI task was not limited to the areas involved during the ankle dorsiflexion but also included parietal and premotor areas more compatible with a real gait behavior. No specific areas were identified for the AO task as compared to the MI task, only a narrower network that did not include the MLR and parietal areas important for sensorimotor integration. We gave a possible interpretation of the results: the internally generated pace defined by MLR could have been bypassed by the presence of the actor, imposing their external pace to the observer. This interpretation appears to be consistent with the positive results obtained for Parkinson disease patients with freezing of gait, using rehabilitation protocols based on the use of AO.

Chapter 4 will propose the comparison between the 22 healthy subjects and a matched group of 22 candidates for TKA who were involved in the same fMRI protocol presented in Chapter 3 to test whether the peripheral limitation due to osteoarthritis would be reflected on the patients neural activations, as a process of maladaptive plasticity. The data showed reduced activations for the patients in absence of compensatory systems. The network singled out by the comparison for the MI condition identified cortical and subcortical areas. Especially the parietal cortex, the precentral gyrus and the putamen identified the larger clusters. The

latter appeared to be especially important: according to the literature on stroke patients, lesions to the putamen are responsible for defining the level of asymmetry of gait, a type of deficit consistent with the limitation reported by our patients. The areas reported for the imitation task were the postcentral gyrus and parietal cortex, relevant areas to movement control and multisensory integration. It appears that osteoarthritis was able to influence the neural responses of our TKA candidates. Moreover this modulation was reported for both the imagery and imitation condition. Also our results showed that the patients activates some of the areas related to motor imagery in the healthy elderlies during the tasks requiring to execute a real ankle dorsiflexion during the stimuli presentation.

I conclude with **Chapter 5**, discussing the data obtained to validate the use of AO and MI as possible rehabilitation tools.

CHAPTER 1 - *General introduction*

In this chapter I will introduce the topic of gait control and its study, which have become relevant in the cognitive neuroscience field. Through the description of gait neurophysiology, in the first paragraph I will show how the concept of gait control has evolved: starting from the initial interpretation of simple automatic behaviour based on spinal reflexes to a complex system of sensory and motor information integration modulated by an extended cortical network. In the second paragraph, I will continue by briefly introducing the literature on gait control and its relation with the cognitive domain, with regards to the changes across the life span, to underline why the use of fMRI has become relevant to better understand the control of locomotion. Finally, in the last three paragraphs, I will present the methods involved in the fMRI studies to allow the investigation of gait control.

1.1 The neurophysiology of gait

The ability to walk defines one of the most common actions in our everyday life. Walking upright is one of the specific abilities of humankind and has an enormous impact on our daily routine, so that it is one of the first indexes considered when checking an elderly person's health, and a distinctive parameter for the definition of a person's autonomy.

The weight of gait disturbances on the public health system is high and reflects the frequency of gait disorder and impairment occurrence due to different causes.

Gait has traditionally been considered a mere ballistic and automatic behaviour, as the title of McMahon's classic text "Muscles, Reflexes and Locomotion" [1] effectively summarizes.

This view is a consequence of some aspects of the locomotion control: as a matter of fact already 100 years ago Graham Brown [2] demonstrated that the cat spinal cord is able to create the locomotion rhythm even if isolated from the higher centers and the afferent feedback.

The rhythmic alternations of reciprocal activity of flexor and extensor muscles are actually governed by the spinal cord. Here the so-called "half centers", interneurons defined as "central pattern generators" (CPGs), carry out the task to inhibit and excite the population of motor neurons responsible for muscles activity [3, 4].

The concept of CPGs has evolved along the years due to the fact that the rhythmic organization of locomotion is only apparently simple. The models representative of locomotion control had to include the different parallel sub-processes necessary to coordinate the activation of flexor and extensor motor neurons, where the main problem is the coexistence of different simultaneous inhibition and excitation processes. One theory to solve this problem was the existence of modules

defined “unit burst generators” (UBG) [5], that controlled subsets of motor neurons, able to work independently. Similarly new models involving adjunctive interneurons that cooperate in a single CPG have been proposed [6, 7]. The single GPG should distribute to the organization of the rhythm and the pattern of locomotion between multiple nodes. The idea that a single CPG could be responsible for both the rhythm and the pattern organization of the movement appeared to be reductive: for this reason, a model with multiple CPGs has been proposed, dividing the different roles between a “rhythm generator” (RG) and a “pattern formation” (PF) [8].

These models were also better fitting from the point of view of the interaction between the ascending sensory information from the skin and peripheral system, involved in the on-line feedback regulation of the movement, and the descending information, from the brainstem, the cerebellum and cortex. [9] (Figure 1.1)

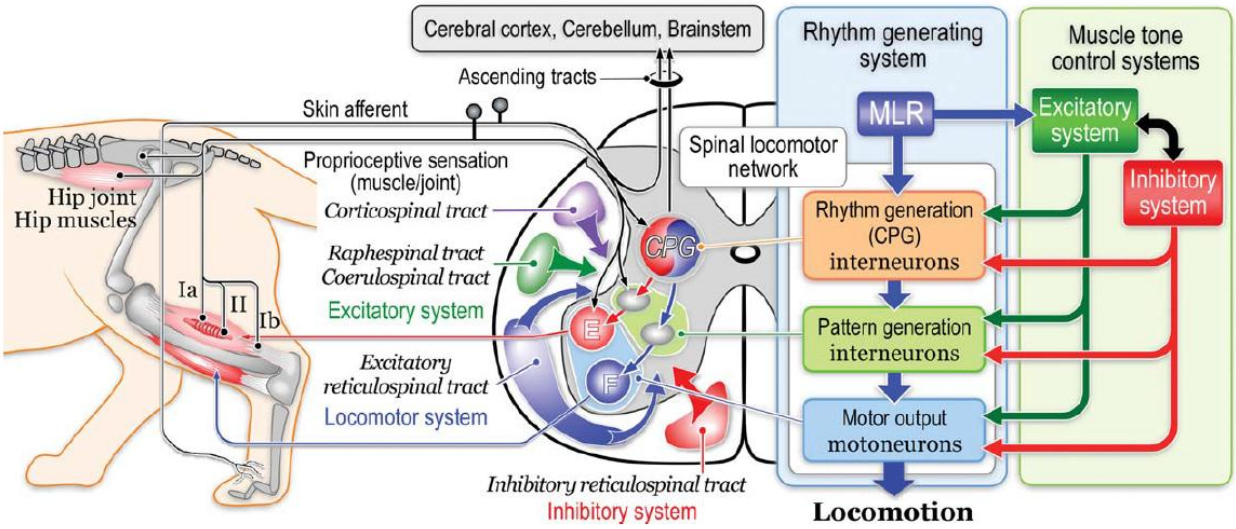


Figure 1.1 Organization of the spinal cord motor control in cats as illustrated by Takakusaki (2013); central pattern generator (CPG), mesenchephalic locomotor region (MLR)

The idea that the spinal cord could be the only structure responsible for gait control was dismissed long ago. As a matter of fact, the spinal cord is only the final step of locomotion control: if the CPGs represent the centres for the rhythmic alternations of muscles flex-extension, it is the brainstem that makes controlling it possible through the monitoring of muscle tonicity and the postural adjustments. The brainstem also has an important role for locomotion initiation [9, 10].

Brainstem control of gait.

The ability to control different types of locomotion in the vertebrates was identified in the so-called “Mesenchephalic Locomotor Region” (MLR) located at the border between the midbrain and hindbrain [11]. The MLR is linked to several superior areas and absolves different functions, redirecting the different ascending and descending information. The MLR is composed by three

nuclei, the cuneiform and subcuneiform nuclei (CuN, SCuN) and peduncolopontine nucleus (PPN). The CuN, considered a sort of defensive system, is responsible for the fine control of locomotion and cardiovascular and analgesic responses to painful or threatening stimuli and receives inputs from other structures generally involved in "fight or flight" reaction, such as the medial amygdala, the medial hypothalamus and the periaqueductal gray [12]. The PPN is considered the sum of two different functional modules: the dorsal PPN (d-PPN), involved in locomotion control, and the ventral PPN (v-PPN), a muscle tone inhibitory system [9].

Excitatory and inhibitory pathways within the brainstem

The brainstem pathways are divided in two main systems with reciprocal function: an excitatory and the inhibitory muscle tone system. The excitatory system reprojects the information from the basal ganglia, globus pallidus (GPi) and pars reticulata of the substantia nigra (SNr) to the MLR (consisting of the d-PPN and the CuN). The MLR indirectly activates the CPGs in the spinal cord through three tracts deriving from the raphe nuclei (RN), the locus coeruleus (LC) and the ventral part of the medullary reticular formation (v-MRF). This excitatory system works in concert with two other locomotor regions, located in the subthalamus (SLR) and the cerebellum (CLR) (see Figure 1.2). On the other hand, the inhibitory projections of GPi and SNr reach the PPN, cascading to the pontine reticular formation (PRF) and subsequently the dorsal part of the MRF (d-MRF). The final nodes of this pathway is the lamina VII of the spinal cord. Similarly to the projections of SLR in the excitatory system, the hypothalamus is involved in the inhibitory process (see Figure 1.2). [9, 13]

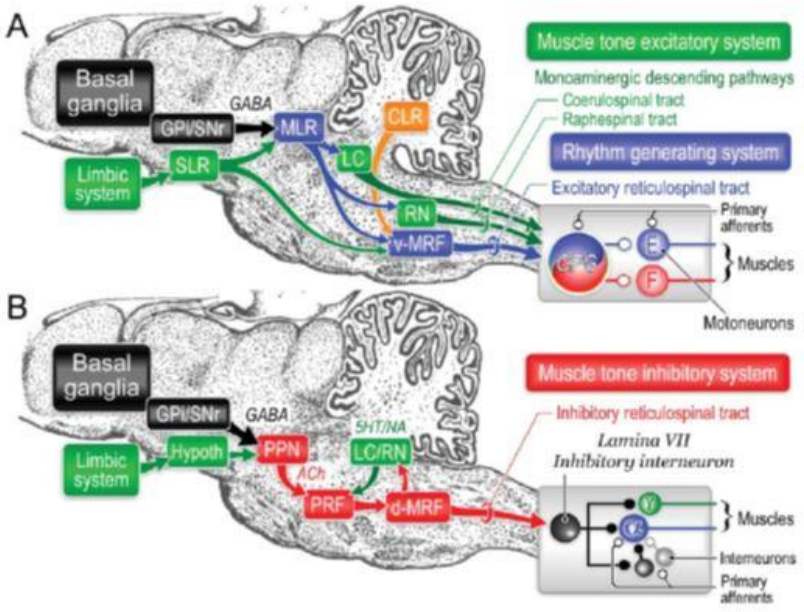


Figure 1.2 Brainstem model for locomotor control in the cat as proposed by Takakusaki (2013). Mesenchephalic locomotor region (MLR); globus pallidus (GPi); pars reticulata of the substantia nigra (SNr); raphe nuclei (RN); the locus coeruleus (LC) and the ventral/dorsal part of the medullary reticular formation (v/d-MRF); subthalamic locomotor region (SLR); cerebellar locomotor region (CLR); pontine reticular formation (PRF).

Cerebellar control of gait

The level of locomotion control described till now represent only the direct modulation of the movement effectors. The role of the lower areas, such as the spinal cord and the brainstem, is not sufficient to explain gait control: in fact, gait is the result of multiple interactions between the cortical and cerebellar areas. The cerebellum integrates the high level motor program, through the cerebro-cerebellar projection, and the automatic loops from the spino-cerebellar ascending pathways to coordinate the different motor segments involved in locomotion. The expression of this

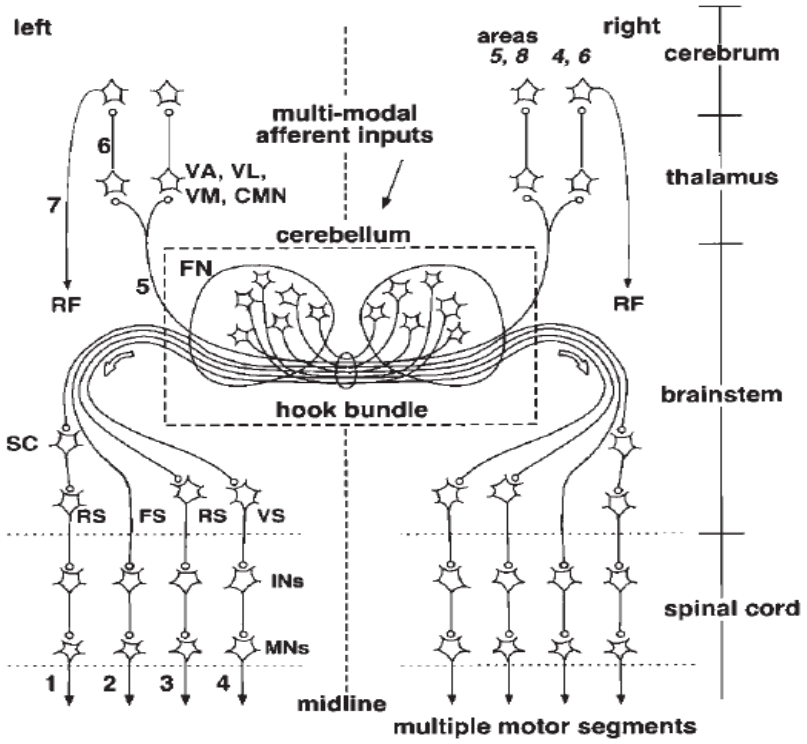


Figure 1.4 Scheme of the ascending and descending pathways as presented by Mori and coll. (2004); ventroanterior (VA), ventrolateral (VL) , ventromedial (VM) and central medial (CMN) nuclei of the thalamus; fastigio-tecto-reticulospinal (RS), fastigiospinal (FS), fastigio-reticulospinal (RS) and fastigio-vestibulo-spinal (VS) pathways; interneurons (INs) and motor neurons (MNs) of the spinal cord (SC).

higher order motor control could be comprehended by describing gait ataxia, a prototypical consequence of cerebellum damage. Since gait ataxia defines a deficit of the motor program, it does not imply an impediment in execution, but it causes an imprecise modulation of force and velocity in the implementation of a motor scheme that results in a generalized "clumsiness" characterized by widened base, unsteadiness and irregularity of steps, and lateral veering. [14] The CLR is made up by the rostral and most anterior part of fastigial nucleus of the cerebellum (Figure 1.3) [15]. The ascending pathways of the fastigial nucleus project to the ventroanterior (VA), ventrolateral (VL), ventromedial (VM) and central medial (CMN) nuclei of the thalamus. Once it reaches the thalamus, the information is projected to the premotor and supplementary motor areas (BA 5,8,4,6) through thalamo-cortical connections, and from there to the reticular formation (RF) through cortico-reticular descending pathways. On the other hand, the CLR is able to project to the interneurons

(INs) and motorneurons (MNs) of the spinal cord through fastigio-tecto-reticulospinal (RS), fastigiospinal (FS), fastigio-reticulospinal (RS) and fastigio-vestibulo-spinal (VS) pathways.

Cortical control of gait

The last part of locomotor control arises directly at the cortex level with the definition of the network of areas involved in the definition and organization of the motor program. Differently from the subcortical control of locomotion, the role of the cortical areas integrates all the visuo-spatial information on where the movement needs to be executed with the program of how it should be executed. The motor program of the premotor (PM) and supplementary motor area (SMA) is actuated by M1 while receiving sensorial information from S1. The sensorial information is itself an integration of visual, somatosensory and vestibular information organized by the posterior parietal cortex (PPC) and the temporo-occipital cortex in an organized body schema. (Figure 1.5)[9]

The final result is an integrated control of locomotion that allows to define a motor program from the cortex, imposing posture adjustment based on anticipatory organization of the movement that considers both visual aspects of the environment and kinesthetic, vestibular and sensory information. This program is flexible, allowing continuous corrections through the constant cerebellar monitoring based on sensorial and motoric feedbacks from the brainstem and spinal cord.

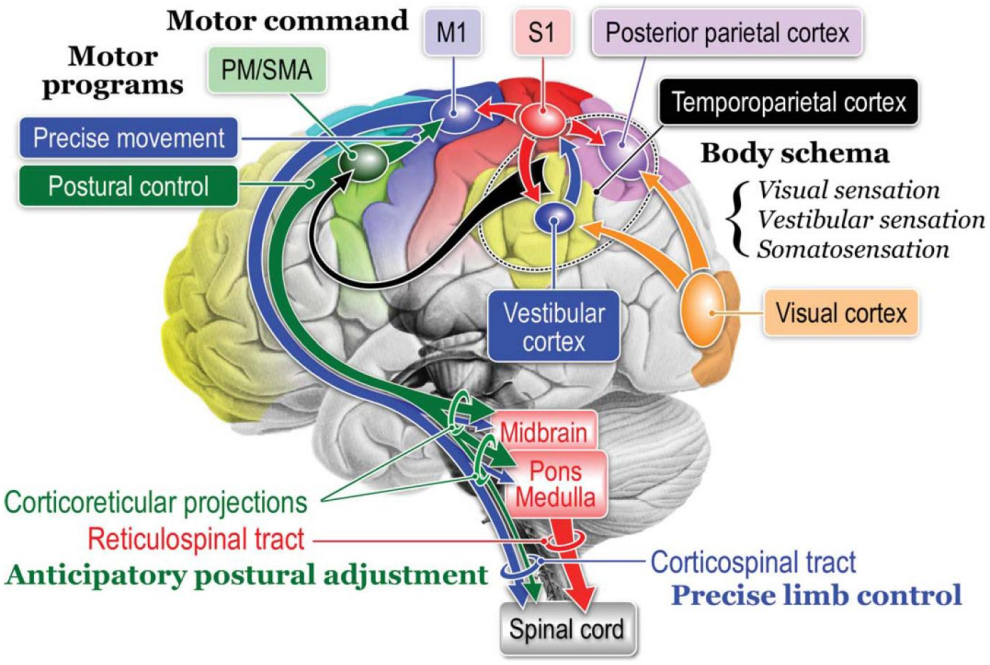


Figure 1.5 Cortical organization of locomotion with its projection to subcortical and spinal afferences as proposed by Takusaki (2013); supplementary motor area (SMA), premotor area (PM).

Evidence of the role of a more cognitive control for gait behaviour has been reported as a consequence of brain damages: "gait apraxia" (see above) [16] has been reported for damages in the supplementary motor area (SMA), or the effect of damages in the premotor cortex (PM) in the adaptation to external changes and cues [17, 18].

More recently, a definition of "higher-level gait disorders" [19] (HLGD, see Figure 1.6) has been introduced to describe locomotor and balance difficulties that cannot be explained by lesions only involving the ascending and descending motor and sensory system, or limited to subcortical or cerebellar damages.

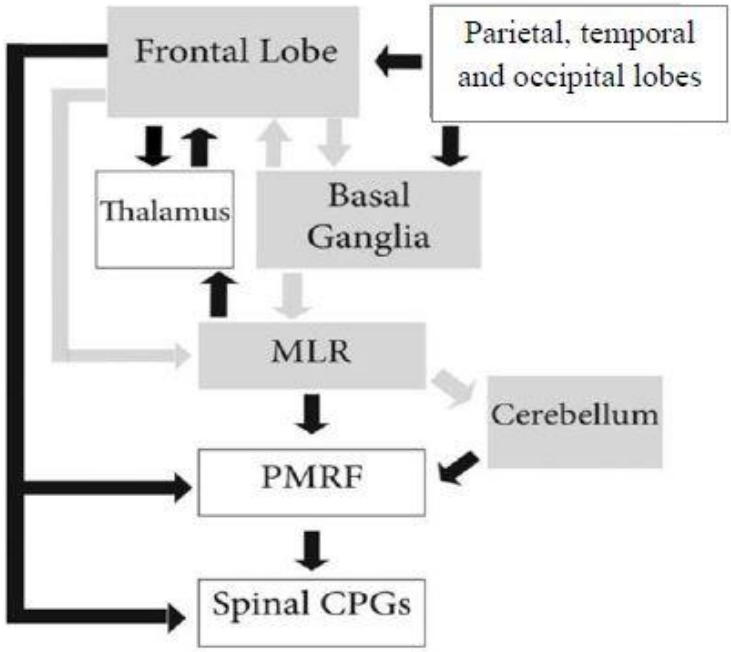


Figure 1.6 Diagram of the neural circuits involved in gait control as presented by Nutt (Nutt 2013); Mesenchephalic locomotor region (MLR); pontomedullary reticular formation (PMRF); central pattern generators (CPGs).

1.2 Gait: development, aging and cognition

The ontogenesis of gait is a continuous process that evolves across the whole life span: from reaching gait stability during childhood to the decline due to aging.

Toddlers begin to "walk" at about 14 months [20]. Their initial locomotion is characterized by extensive trunk oscillation and wide base support, used to compensate their instability [21]. In order to reach balance and equilibrium control, both the somatosensory system and the motor system must develop [22].

The subsequent steps needed to obtain gait stability, that is reached around 9 years of age, are principally two: dropping the initial "high guard" stance, characterized by the upper limbs kept still and high, for the synchronization of the arm movements coupled with controlateral steps [23], and the stabilization of the head movements [24].

From the age of 9, the parallel development of the musculoskeletal system and the cognitive maturation define gait evolution. When speaking of human mature locomotion as "stable gait", we use a term that could equally be referred to different aspects: it could mean a step-to-step repeatable walking [25], a gait resilient to external and internal perturbations [26], or the ability to maintain upright balance during walking [27].

This extended concept of stable gait has led to an evolution of the interpretation of gait disturbances: if musculoskeletal impairments, such as the lower limb muscle reduction, the increasing functional limitation and cartilage loss in joints, have always been considered as the leading cause of gait disturbances [28], especially in case of degenerative osteoarthritis [29], other complex parameters have made their appearance to interpret locomotion. Quantitative analysis of gait (gate analysis see fig 1.5) has introduced new kinematic measures able to detect different profiles between walkers: the step width and stride velocity proved to be able to distinguish old and young subjects, while the variability of stride stance and swings can separate fallers and non-fallers [30]. The kinematics measurement has also allowed to single out parameters, not limited to the leg movement but equally important for gait quality: especially trunk acceleration can compromise gait speed and stability [31] and even predict falls independently from physical performance [32]. The control of trunk acceleration and the upright stability are the result of the integration of different sensorimotor, vestibular and visual information. This means that the decline in vision, peripheral sensation and reaction times involved in aging is directly reflected on gait behaviour: as a matter of fact, aging is accompanied by a variation in step timing, reduced velocity and short step length [27].

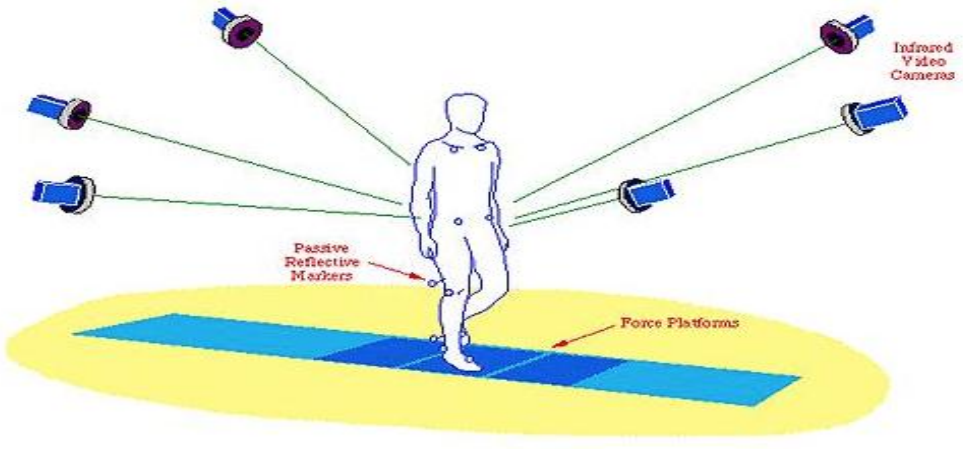


Figure 1.5 schematic representation of the acquisition method of gait analysis.

These results have underlined the importance of integrating the cognitive aspects in the study of locomotion. In the last 10 years, the literature that explored the complexity of gait, both at a behavioural and neurophysiological level, has extended its knowledge to include the cognitive aspects of gait control, integrating the contribution of cognitive neuroscience and neuropsychology. Hausdorff et al. [33], for example, have compared locomotion to simple and complex higher limb movements and found a correlation between different gait measures, such as gait speed and stride variability, and the measures acquired during the organization of the complex motor program needed to catch a moving object with respect to the frequency and variability of a simple tapping task. They also reported a positive correlation between the gait parameters and the neuropsychological tests that measured executive functions (EF).

Similar results have also been reported by Beauchet et al. [34] who propose that gait control could be considered a subdomain of the EF, based on the relation between the stride variability and two neuropsychological tests for EF in a large group of healthy elderly subjects.

The cognitive aspect of gait has gained more importance especially in relation to aging, in relation to both the structural and functional neural alterations. Changes in gray and white matter have been reported with aging, especially in older adults (>65 years): the rule “last in, first out” has been used to underline how the prefrontal and frontal regions, both in the dorsal and orbital portions, are the last brain regions to develop and the first to atrophy [35, 36]. The somatosensory and motor cortices have been reported to be involved in this atrophy with the reduction in the pre and post central gyri gray matter, as shown by voxel based morphometry [37]. A similar reduction has also been reported for the cerebellum [38]. Changes in white matter have been reported in older adults with special regard for the corpus callosum and its relation to motor control, with disproportion for the anterior fiber bundle with respect to the posterior one [39]. The decrease in white and gray matter is reflected in compensatory strategies for motor tasks that require more cognitive resources that are however limited too [40] (Figure 1.7). Due to the neural depauperation and reduced cognitive resources, motor control in aging is characterized by a more widespread activation of the neural cortex with less specific networks, with specific involvement of the prefrontal cortex and the basal ganglia [40] With relation to gait control, Holtzer and colleagues [41] reviewed the literature describing how poor mobility is related with the reduction of gray and white matter volume. Apart from the structural changes due to aging, the authors underline how functional studies could explain the activation patterns of gait control. They also say that even if some knowledge on the neural gait has been acquired, such as the involvement of the frontal and parietal cortices, the basal ganglia and the cerebellum, there is a lack of a consistent literature on the functional neural networks involved in gait control.

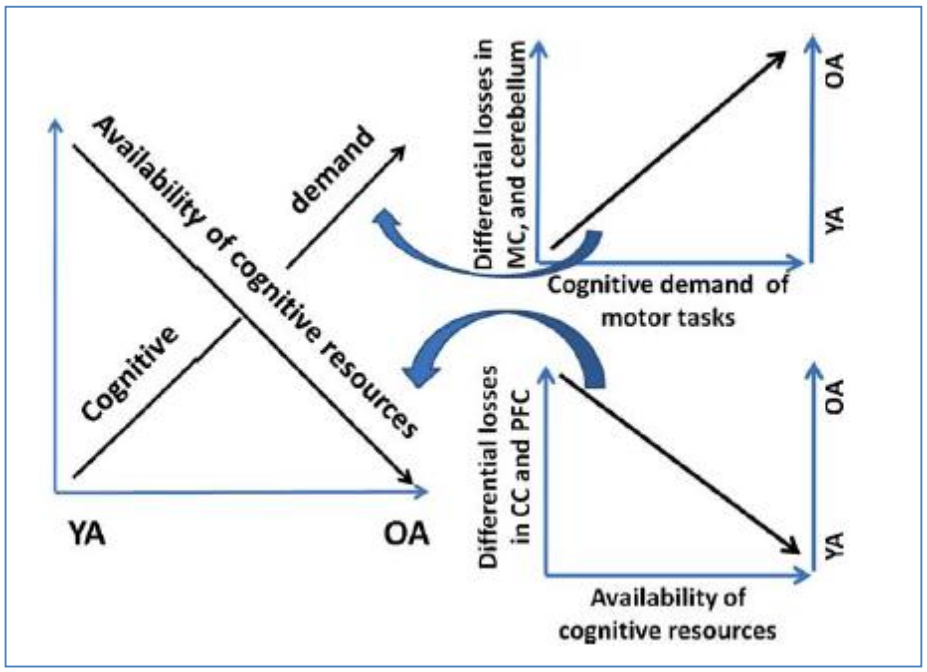


Figure 1.7 The "supply and demand model" framework proposed by Seidler et al. (2010). Due to neural atrophy in the motor cortex and cerebellum, older adults rely more on cognitive resources to accomplish motor tasks; however, the availability of the cognitive resources is reduced due to the prefrontal and corpus callosum atrophy. Corpus callosum (CC); prefrontal cortex (PFC); motor cortical regions (MC); young adults (YA), older adults (OA)

The evolution of the concept of gait control, from the initial limited interpretation as an automatic behaviour to the actual interpretation as a cognitive domain, has made it necessary to carry out a deeper investigation of the neural patterns involved during active locomotion. The biggest issue, as Holtzer et al. also underline, is the physical limitation of cognitive neuroscience methods. Noteworthy are the studies of Miya and colleagues [42], that tried to investigate the neural control of locomotion the near infrared spectroscopy (NIRS), a methodology that allow registration during a movement execution an the study of la Fougere [43] that asked to its participants to actually walk before the a PET scansion, marking the consume of for the active movement. One of the most common methods to study task related neural activation patterns is fMRI, but the fMRI context is incompatible with a task that involves walking. This apparently unsolvable problem resulted in the creation of alternative methods to study gait control. In the next paragraph, I will present how the use of fMRI has been introduced in the study of gait control.

1.3 Implementation of gait related tasks in the fMRI context

The use of fMRI has been introduced in the study of normal and pathological gait to obtain information on the neurophysiology of gait. Due to the physical limit of the environment of the instrumentation, however, it has been necessary to find valid alternatives to real locomotion to study the explicit movement in the experimental conditions. The principles followed by different authors

were to find simplified actions compatible with the fMRI environment that could however reflect an intrinsic aspect of gait, and activated the areas involved in the gait brain network. Dobkin and coll. [44] conducted a study to validate the use of the ankle dorsiflexion in 12 healthy subjects and tested whether the same task could be used as a parameter to estimate the capacity for near-term and ongoing reorganization-associated rehabilitative gains in gait recovery in hemiparetic patients. In the healthy subjects, the authors compared the regions involved both in the voluntary and passive ankle dorsiflexion and described a network that involved controlateral S1, M1 and SMA with lower effect in SII bilaterally for the voluntary movements, in contrast with the opposite scenario for the passive one. For the voluntary ankle dorsiflexion, activations were also reported in the ipsilateral thalamus, basal ganglia, and dorsolateral prefrontal cortices, and ipsilateral cerebellar hemisphere. They then found positive correlations between the brain activation and the measures for physical gait recovery in 4 hemiparetic patients. Ciccarelli and coll. [45] reported a major activation of the supplementary motor area (SMA) in the active movement compared to the passive one, which underlines a major role of areas involved in motor preparation like the ispilateral M1. These results are concordant with the further comparison carried out by Francis and coll. [46] between electrically stimulated movement and active movement, with greater activation for the latter in the SMA, dorsal controlateral premotor rostral part (PMdr), ventral controlateral rostral part (PMvr) bilaterally, dorsolateral prefrontal cortex, the cingulated motor area (CMA), and the ipsilateral cerebellum IV. Due to the reported brain networks involved, it appears that voluntary ankle dorsiflexion leads to the activation of areas responsible for motor planning, execution and visuo-motor coordination. A direct comparison of the output for ankle dorsiflexion and the BOLD response has been proposed by MacIntosh and coll. [47], who measured the flexion degrees through electromyography (EMG) to match the increase of the angle with the increase of BOLD signal, obtaining a parallel trend between the two. Newton and coll. [48] compared different movements (ankle dorsiflexion, ankle plantarflexion, and knee extension) using a custom-made apparatus compatible with the fMRI to observe the difference between the different muscular activation at a brain level. Even if they found greater activation in SM1 for the knee extension, the substantial overlapping of the region activated during the different movement corroborates the idea that the isolated ankle dorsiflexion could be considered a good representative of a more extensive movement and therefore a good representative of gait.

Apart from real execution, different approaches involving mental motor imagery and action observation have been used to define gait related tasks in the fMRI context. These approaches will be described in the following paragraphs.

1.5 “The motor imageries” and their application

Motor imagery (MI) is a mental state during which movements are mentally evoked and rehearsed without overt actions [49]. There is a consistent amount of literature on the relation between MI and action execution, however since it’s principally focused on the upper limbs, I will introduce the general concept and characteristics of MI before moving to the gait related studies.

MI is viewed as a window on the motor system, specifically on the motor control and motor organization, and on the relation between action execution (AE) and its imagined counterpart, and it has been defined as “mental rehearsal” or “mental simulation” [50-52]. In the last two decades, the concept of MI has evolved thanks to different neurophysiological and behavioural methodologies and, as recently underlined by Hanagawa [53], it would be more correct to talk of “motor imageries” (see Figure 1.8). This definition reflects the multiple aspects involved in MI, including different stages of motor control, the conscious or unconscious access to MI, or the sensory modality by which MI is driven by.

MI has been behaviourally measured through mental chronometry, which is based on the comparison between the time needed to imagine an action and the time needed to effectively execute it. Mental chronometry has been a useful tool to simply measure the complexity of an action, based on the isochronism between AE and MI, and to investigate aspects of MI at an implicit and explicit level, using different types of instructions to perform a task.

In explicit MI, subjects are directly asked to imagine themselves while executing the required actions [54] and to focus on kinesthetic bodily sensory information by taking a first-person egocentric perspective. In literature, the studies using the mental chronometry paradigm in explicit MI conditions have reported how physical constraints in AE are reflected in the MI tasks due to the principle of isochronism [55, 56].

On the other hand, in implicit tasks, the MI process may be triggered without explicit reference to the concept of MI during “prospective action judgements” [57], Laterality Judgement Task (LJT), where subjects are asked to decide whether the hands portrayed in a picture (rotated at different angles) are the left or the right one.

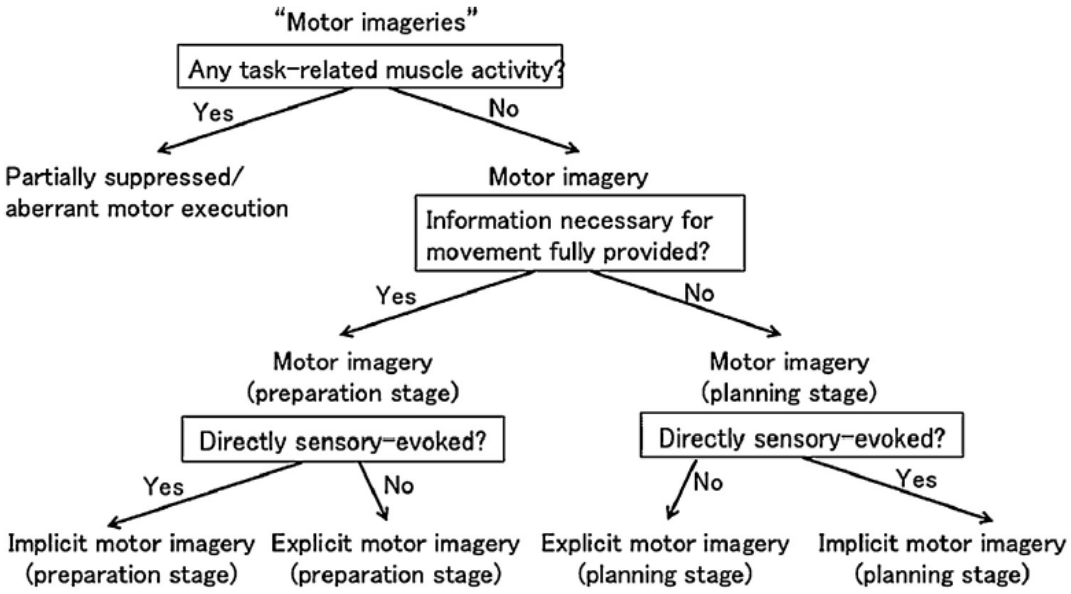


Figure 1.8 Hanagawa (Hanagawa 2015) model for complete or partial MI motor planning and for explicit and implicit MI.

It is believed that, during this task, subjects unconsciously simulate a mental rotation of their own hand to match the position of the depicted hand stimulus, hence producing "motorically driven perceptual decisions" [58, 59].

The results obtained using the LJT paradigm as a measure for MI reflected how the reaction times (RTs) are influenced by the biological constraints or the specific motor impairments that affected the subjects in real execution [60, 61].

Other evidence of the relation between AE and MI were reported using transcranial magnetic stimulation (TMS) combined with electromyography (EMG), to obtain evidence of a pre-activation of motor pathways during motor imagery tasks [62, 63].

Findings from imaging techniques such as fMRI and PET studies have reinforced the bond between MI and AE, showing partial overlapping between the neural networks involved ([64] and Figure 1.2 for a systematic meta-analysis on the neurofunctional effects associated with MI). The "motor imageries" concept finds supports in imaging data: the implicit and explicit nature of MI or the level of complexity of the MI task are expressed by different networks of functional activation. The same goes for the strategies used to perform an MI task, that differ based on the sensory modality that drives the rehearsal. As initially defined by Hall and colleagues [65], we talk about visual MI (VMI) and kinesthetic MI (KMI): VMI involves imagining seeing yourself performing the task, while KMI involves the feeling that the actual task performance produces. This distinction is crucial, since the strategy used reflects how an MI task is directly connected to AE. As reported by the ALE analysis by Hetù et al. (Figures 1.9 , 1.10), the shared network of the motor imageries, and especially the

motor areas, are generally activated during KMI tasks, and give important clues on how MI protocols should be implemented to obtain information on AE.

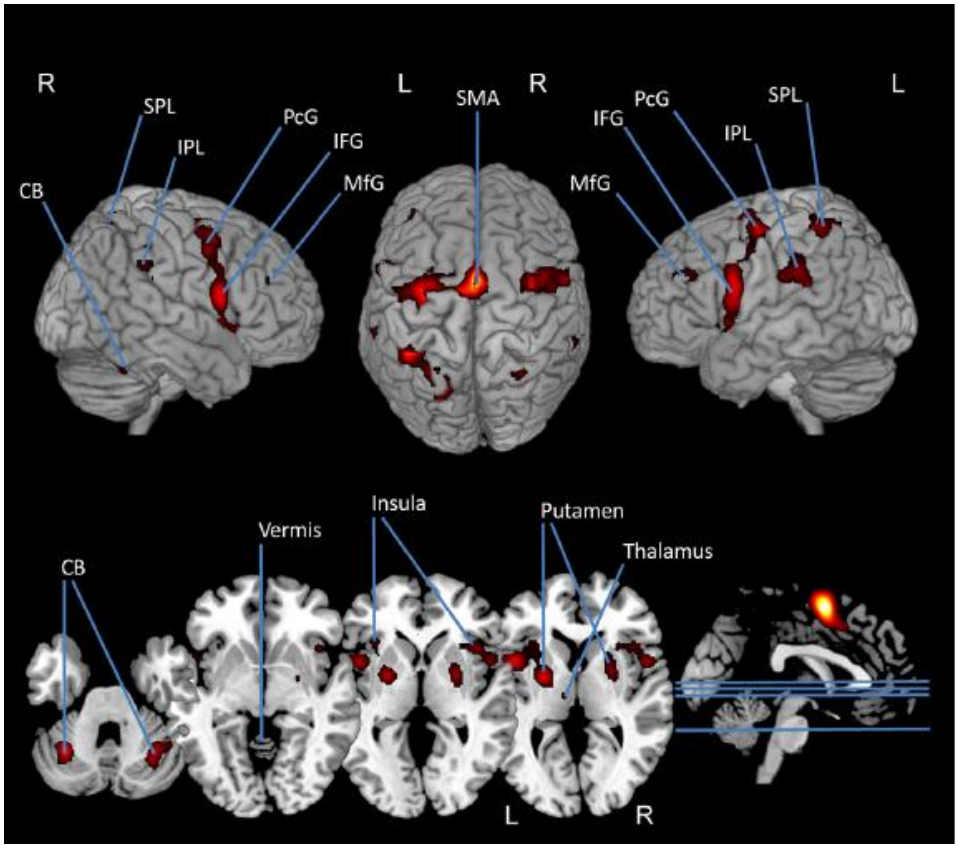


Figure 1.9 Shared neural network for MI tasks as presented by Hetù et al. (Hetù et al. 2013)

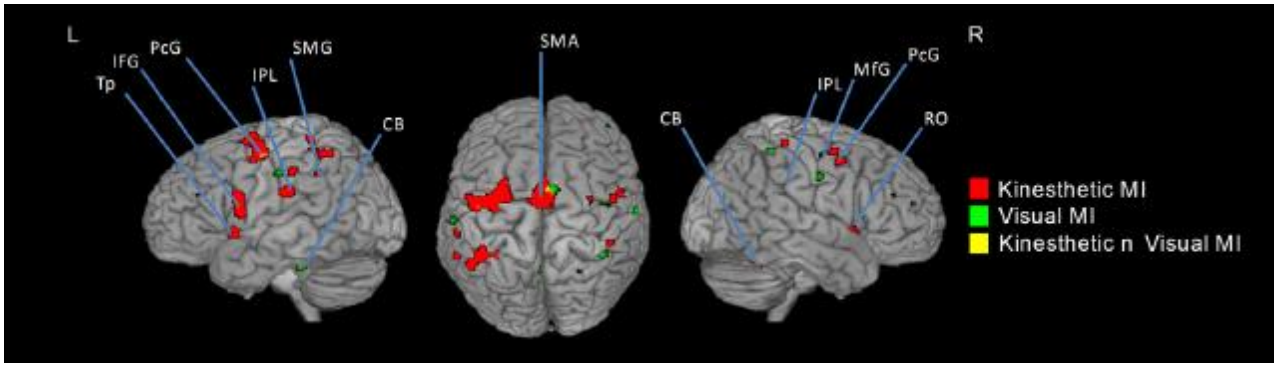


Figure 1.10 Differences between KMI and VMI as presented by Hetù et al. (Hetù et al. 2013)

MI protocols are relevant for AE due to the principle of functional equivalence: the similarity between MI and AE, as behaviourally and functionally observed, implies that if the access to AE is somehow limited, MI could be used as a backdoor for the motor rehabilitation. Ridderinkhof and colleagues have recently proposed a neurophysiological model that specifically underlines the shared network between AE and KMI (Figure 1.11; [66]).

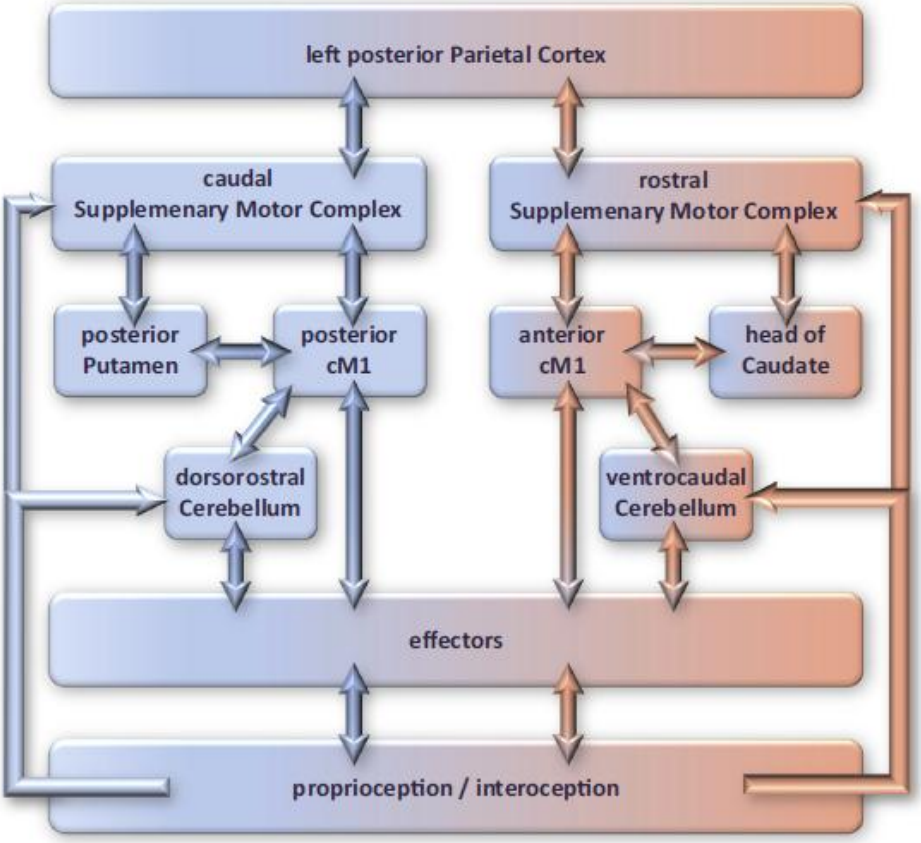


Figure 1.11 Ridderinkhof (Ridderinkhof et al. 2015) model for AE (blue) and KMI (red) shared networks

Due to their different properties, KMI and VMI have a different impact on the motor recovery as measured by corticospinal excitability: Stinear et al. [67] have shown how only KMI tasks are able to evoke motor evoked potentials (MEPs), while the VMI effect is equal to a visual imagery task unrelated to movement. The knowledge acquired in the experimental context with a healthy population has been used as a tool to explore how physical constraining or motor reduction could influence the motor representation in different clinical contexts, especially for neurological disorders. Deficits in MI tasks have been reported for patients with spinal cord injuries [68], Parkinson's disease [69], and stroke patients (for a review, see Zimmermann-Schlatter [70]et al. and Di Rienzo et al. [71]). Rehabilitation protocols have been created for the same types of patients described above, using MI to promote motor recovery while avoiding the fatigue and pain of physical exercise caused by the limited mobility of these patients. (For a review, see Braun al. [72];

Malouin et al. [73]). The use of MI protocols has recently been extended to non-neurological pathologies [74, 75].

The rehabilitation protocols still need standardization: the patient samples reported in literature are generally small, the protocol definition appears to be incoherent and not completely standardized, making it difficult to correctly apply a therapy based on KMI or MI. The absence of standardized measures also limits the ability to report the effectiveness of a treatment. [72, 76].

1.4 Gait and MI

Literature on MI has greatly increased in the last two decades. The knowledge on MI is not equally distributed in the literature and substantial differences can be observed by simply selecting the effectors involved, instead of the broad general concept: the number of studies that investigate the effects of MI for lower limbs are consistently less numerous than those related to the upper limbs.

MI leg- or gait-related tasks are needed cause MI is not a unique ability and the neural networks involved in MI are divided in the premotor and the motor cortex following the same somatotopic organization of AE [77] . Support for the somatotopic organization for imagined tasks has been found by Carrillo-de-la-Pen et al. [78] using event-related potentials (ERP): the same lateralized readiness potential (LRP) has been reported for both executed and imagined action during a task with congruent (target and flanker arrowheads pointing in the same direction) and incongruent (target arrowheads pointing in the opposite direction to the flanker arrowheads) trials where participants had to respond using the left or right hand, or the left or right foot based on the location of the target, in two separate sessions.

The reduced information on lower limbs MI concerns both experimental and clinical studies. In Hetù's meta-analysis [64], previously described, the author himself acknowledges that the neural networks for the upper limb MI and the neural network for general MI are nearly the same due to the limited presence of MI studies on the lower limbs. The author also underlines the difficulty in identifying subcortical circuits for gait, and the physical limit of the fMRI context to compare the leg AE and its MI counterpart (see the previous paragraph).

Upper limbs studies are also the majority in rehabilitation literature: the treatment of gait deficits has only recently gained some interest for patients with Parkinson's disease [69], post-stroke patients [79, 80] or spinal cord injury patients [81]. However, these studies generally include small samples, with few exceptions [80].

Another aspect that should be integrated in the MI literature is the application of MI protocols to orthopedic conditions. Based on the functional equivalence and the behavioural data in literature about the effect of physical constraint in the MI implicit or explicit task, the application of a MI rehabilitation in the orthopedic context should be as relevant as for neurological disorders.
There are no rehabilitation studies in scientific literature proposing a protocol based on MI for gait recovery in the orthopedic context. We can therefore conclude that, at the moment, there is a lack of knowledge about the neural processes involved in gait MI and its application in the rehabilitation context with specific regard to orthopedic conditions.

1.5 Action observation: neural basis and its application for motor rehabilitation

Action observation (AO) has gained more and more relevance over the last two decades, since the discovery of the so-called "mirror neuron system" [82, 83] in the macaque, a neural system capable of firing both for an executed and an observed action. In macaque the single-neuron registration method as used on the were to investigate the existence of a mirror network. In the human brain indirect evidence showed the existence of mirror neurons has been found in functional imaging studies that have revealed the activation of the likely homologue of the monkey area F5 (area 44 and the adjacent area 6) during action observation [84]. In addition the studies fMRI studies of Kilner [85] and Chong[86] individuate areas that respond both for action and observation in,respectiverly, the inferior frontal gyrus and the right inferior parietal lob. Finally Mukamel has reported evidence of a mirror network in the supplementary motor area (SMA) the hippocampus using the intracranial recording.[87]
Since then, AO has been also studied to identify the characteristics of the observer's intent. Observing something is not always the same action, and the difference is due to the goal of our observation: we can passively observe something, or observe something to imitate it. The intent of our observation reflects in different neural networks ([88] and Figure 1.12 for a systematic meta-analysis of the neurofunctional effects associated with AO for the upper limbs).
The relation between AO and AE has found support in studies using non-invasive brain stimulation methods like TMS to test the effect of AO on the corticospinal excitability: Roosink and coll. [63] reported a facilitation effect on the motor evoked potentials (MEPs) during the active observation of complex movements. Gonzalez-Rosa and coll. [89] have added other evidence supporting the AO relation with AE by recording the neural activity during explicit tasks using electroencephalography (EEG). In their study, the authors pointed out a sensorimotor activation during training based on

AO which also induced a better performance in the execution of the task compared to a baseline condition. They also reported that the EEG activity during AO was predictive of the motor error during execution, as shown by the kinematics acquisition.

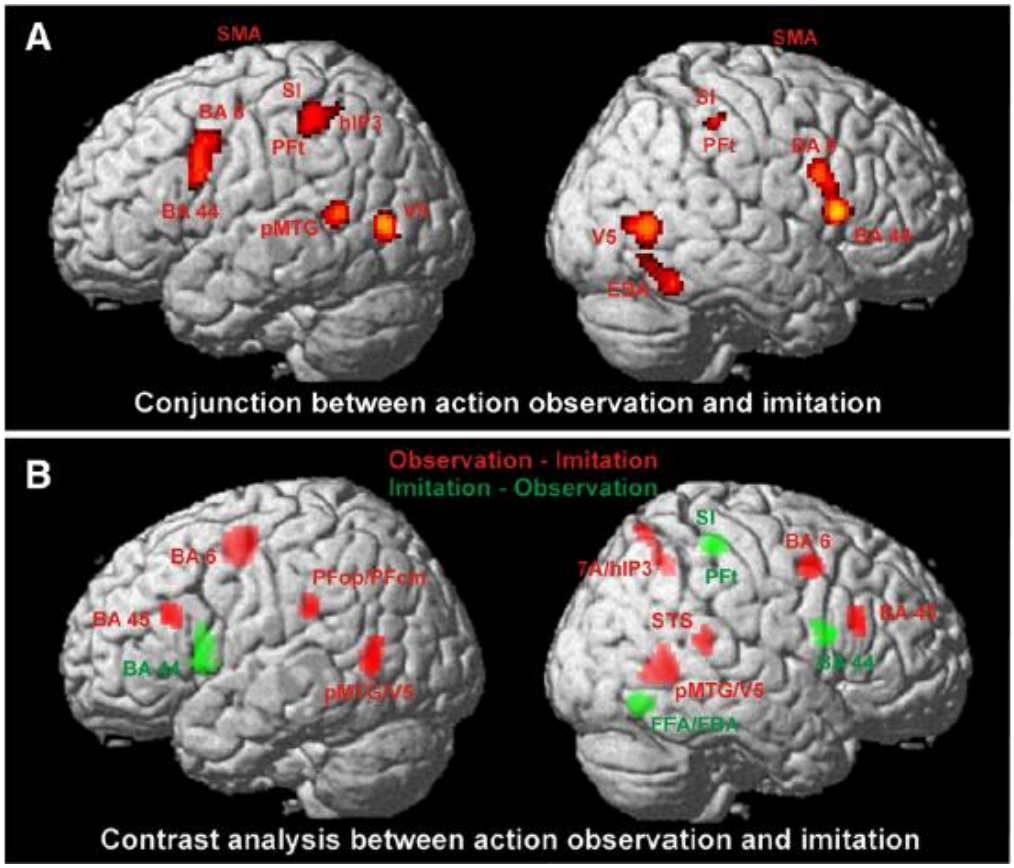


Figure 1.12 The network resulting from the ale metanalysis for imitation AO and passive AO for upper limb movements presented by Caspers et al. in 2010.

Similarly to MI, AO has also been proposed as a tool for motor rehabilitation: AO was used on stroke patients [90-92] and Parkinson's disease patients [93]. Moreover, it was also tested on orthopedics patients [94] . The data obtained by these researches reported a positive effect due to the integration of AO rehabilitative protocols. AO and MI have been frequently compared both to find shared aspects, as proposed by Case et al. [95], who reported a similar neural involvement common to AE, AO and MI, or to try and demonstrate a better effect of AO over MI on the motor recruitment using, for example, EEG [89] and kinematics methods [96].

An important shared aspect of AO and MI is the lack of studies that are related to the lower limbs, exception [97, 98], that will be later discussed in Chapter 3. The understanding mechanisms differentiating AO and MI are limited, even if the principle of somatotopization of the motor and premotor cortex appears to be less clear in the case of AO [77] .

The comparison between AO and MI has been extended to their effects in rehabilitation, generally with the aim of determining which one should prevail as a rehabilitation tool. However, Vogt et al. [99] presented a different approach: after reviewing the rehabilitation literature on AO and MI in isolation, the authors reported the effect of neurofunctional activation in the motor system during tasks that combine and integrate AO and MI, proposing that the integration of the two tools could lead to a better effect on motor recovery.

CHAPTER 2 – *The effect of gonarthrosis on motor imagery: a behavioural study*

In this chapter I will present a behavioural study designed to explore the performances of group of candidates for total knee arthroplasty (TKA) in an executed and imagined gait related task as compared to a group healthy subjects. Moreover we were interested to test whether the pure peripheral pathology of our TKA patients would interfere equally on both the executed performances and their imagined counterpart, as measured by mental chronometry. The focus of the study was to evaluate the impact of the functional limitation caused by the gonarthrosis on a series of tasks involving the lower limbs, including the timed up and go test, which is a clinical measure that was performed both explicitly and during imagination [100, 101]. Due to the task-dependent variability of mental chronometry, we constructed four different tasks, that involved both the upper and lower limbs, controlling for the level of complexity of the tasks and the limb specificity's effect.

2.1 Introduction

Motor imagery (MI) has been defined as "mental motor rehearsal" (see Chapter 1). It implies to re-evoke the acquired information on our ability to execute an action and re-elaborate it to imagine the repetition of the movement. Thus, imagined movement should respect the biological physical constraints that limit the range and possibility of real execution [49, 57, 102]. Furthermore the ability to mentally evoke an action cannot be defined as a static process but as dynamic one, that should keep updated on our physical conditions and take into account how an action has been executed or could be executed based on our experience. For this reason, the MI ability reflects the differences between new and experienced movements [56]: this applies for instance to the differences between professional athletes, who are more used to prepare and execute a specific motor program, and amateurs [103]. The updating should also reflect the reduction of physical functionality caused by pathological conditions, be them of neurological nature [60], temporary immobilization and disuse, or amputation [104]. However, the relation between action execution (AE) and MI is not a stable: the level of difficulty of the motor program involved in an action could influence the ability to imagine it. These differences could be reflected in an underestimation of the time needed to perform the task during the mental rehearsal, caused by a lack of accuracy to reconstruct the motor sequences [105, 106] and to define the correct time constraints and dynamic phases of the movement [107-109]. The opposite scenario could also occur in case of an increase in the complexity of the movement. An action could appear too attention-demanding, or involve many

coordinated movements, or be influenced by environmental constraints, and this could lead to an overestimation of the time needed to execute it during the mental rehearsal [110, 111].
The comparison of MI and AE for walking, among the various actions reported in literature, has generally reported good level of isochrony [56, 112-114] with some exception [115].

2.2 Aim of the study

In this study, we compared the executed and imagined performances of a group of candidates for total knee arthroplasty with a group of healthy controls in a gait related executed and imagined tasks to evaluate the possible effect of the functional limitation caused by a pure peripheral limitation, in both conditions, using the mental chronometry as parameter. The principal topic of the study was the ability to walk but, since gait is an articulated motor program, we created two tasks related to the lower limbs, divided for level of complexity. We also defined two matched control tasks, involving the upper limbs, to effectively test the specificity of the influence of the functional reduction only for tasks involving the lower limbs, our target. The chronometry of both the executed and imagined performances was acquired and compared between the two groups to obtained information on each task separately rehearsal of the movements. Then to measure the level of isochrony for the two groups a second analysis was executed on the differences, expressed in seconds, obtained by the subtraction "imagined minus executed" of the time needed for each task.
The choice of the osteoarthritis as a model of pure peripheral limitation in a group of neurologically healthy subjects has been driven by the severe impairment caused by this pathology in daily life activities. As much as this may surprise the reader, osteoarthritis is considered the single most important cause of disability and handicap in Western industrialized countries [116-119]. It is a major cause of musculoskeletal pain, and daily life activities are reduced due to severe functional limitation that increases for specific actions such as kneeling, stair climbing, walking for long periods of time, or driving, and the movement involved in dressing up or taking care of personal hygiene.
Due to this invasive interference in daily activities, osteoarthritis has become an important community healthcare burden in lost time at work and early retirement, with significant health and welfare costs [120, 121]. The knee and hip joints are the most frequently involved in osteoarthritis [122, 123] and hip and knee osteoarthritis has been identified as one of the five diseases responsible for the greatest proportion of physical disability in non-institutionalized elderly men and women [124].

TKA is a surgical intervention for severe gonarthritis, i.e. the specific osteoarthritis involving the degenerative loss of cartilage and bone deformation of the knee joint, and it involves the complete substitution of the bone knee joint with a prosthetic one. It is considered to be a successful treatment, in view of the good recovery after surgery[125].

2.3 Materials and methods

For this study the sample was composed by two groups: 25 healthy subjects (women= 10, age 66.79 ± 6.97, education 13.54 ± 4.14 years) and 22 patients candidates to a TKA (women= 14; age 69,5 ± 8,47; education 8,55 ± 3,5). TKA candidates were recruited from the Orthopedic medical division of the IRCCS Istituto Ortopedico Galeazzi, Milano. The leg involved in the surgery was the left one for all the patients selected.
All the participants had to sign a written consent in accordance with the ethical standards of the 1964 Declaration of Helsinki and had to comply with these inclusion/exclusion criteria:

- Absence of previous neurological or psychiatric diseases
- All participants must be right-handed
- Absence of relevant impairment of gait ability (only healthy controls)
- Compatibility with fMRI environment (absence of metallic parts, pacemaker, claustrophobia, etc.)
- Age ranging between 50 and 80 years

All participants were right-handed as confirmed by the Edinburgh Handedness Inventory [126] and reported normal or corrected-to-normal vision. A brief neuropsychological battery has been used to clinically test the subjects' cognitive integrity. The battery included the Mini-Mental State Examination (MMSE) [127], a test providing a summary index of cognitive functioning, and the Raven's Coloured Progressive Matrices [128], to obtain a measure of abstract reasoning independent from the cultural level. No participants displayed a neuropsychological profile compatible with cognitive impairment. All the participants had no previous knowledge about motor imagery and completed the Vividness of Movement Imagination Questionnaire [129] a self-report questionnaire on explicit motor imagery abilities, administered to test their level of MI abilities.
The patients and the healthy subjects were matched for age (U= 227,000; Z=-1.025 ;p= .305), while the education level resulted significantly different (U=106,500, Z=-3679, p= .000), so the possible influence of the educational level has been considered in the analysis (see the analysis and results

paragraphs). The experimental protocol was approved by the ethics committee of the IRCCS Istituto Ortopedico Galeazzi, Gruppo San Donato. The mean values and standard deviation of the groups are reported in the table below (table 2.1)

Table 2.1 descriptive data for the two groups. Patients (PT); healthy subjects (HS)

		age	education	MMSE	Raven	VMIQ 1st	VMIQ 3rd
PT	mean	69.5	8.55	28.7	32.64	51.14	55.38
	sd	8.47	3.5	1.31	3.88	14.19	19.47
HS	mean	66.79	13.54	29.05	33	47.68	46.68
	sd	6.97	4.14	1.5	3.8	18.05	18.85

Experimental tasks

Four tasks were created to test both the executed and the imagined performances of our subjects: two tasks for the lower limbs were created, based on the level of complexity, and compared to two matched tasks for the upper limbs, as a control. The order of administration has been randomized.

A) **Simple task for the lower limbs:** the subjects were asked to move one leg at a time back and forth, alternatively, while sitting on a chair, sliding the foot on the ground without ever lifting it. The instructions were to extend the knee till only the heel was touching the floor, and then flex it, leaving only the toe touching the floor. The movement was repeated 5, 2, 3 and 4 times during four consequent series. After every series the subject was asked to mentally rehearse the movement, without moving, concentrating on the kinesthetic sensations perceived during the actual execution. The series for the right and left legs were executed separately.

B) **Complex task for the lower limbs:** the subjects were asked to execute the timed up and go test (TUG) [100]. Sitting on a chair, the subjects had to stand up, walk for three meters to a cone on the ground, turn around it, and walk back to sit down again. Similarly to the previous task, the subjects were asked to execute the TUG twice and then mentally rehearse the same action focusing on the physical sensations perceived during the execution (see

Figure 2.1a,b). During both imagination and execution, the subject had to say "cone" aloud while turning around the cone on the ground, and "chair" as they sat down again.

C) **Simple task for the upper limbs:** this task required the subjects to alternatively open and close their hands [130]. The action was repeated for four series composed of 5,2,3 and 4 repetitions, respectively. After each repetition, the subject was asked to mentally rehearse the same movement, focusing on the physical sensation perceived during the real execution. The series for the right left hand and were executed separately.

D) **Complex task for the upper limbs:** the subject had to execute a movement, defined as "simulated walk", that required to use the index and medium fingers of the right hand as legs and "walk" along a reverse S (see fig 2.2) pictured on an A3 sheet of paper. The action was executed first, and then imagined; both performances were repeated twice. During both execution and imagination, the subject was asked to say "middle" and "end" aloud following the landmark on the picture.

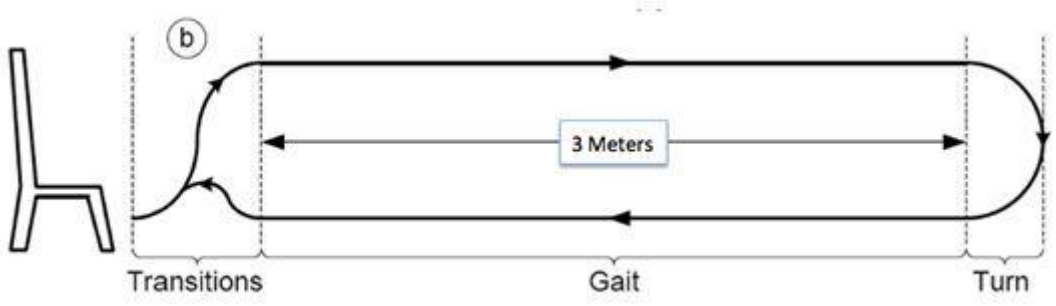


Figure 2.1a a schematic representation of the TUG test

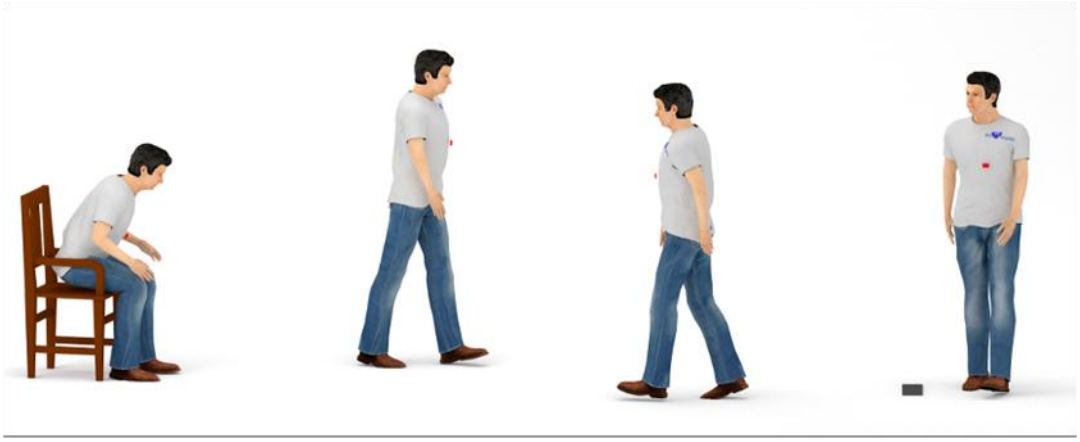


Figure 2.1b depicted simulation of the execution of the TUG test

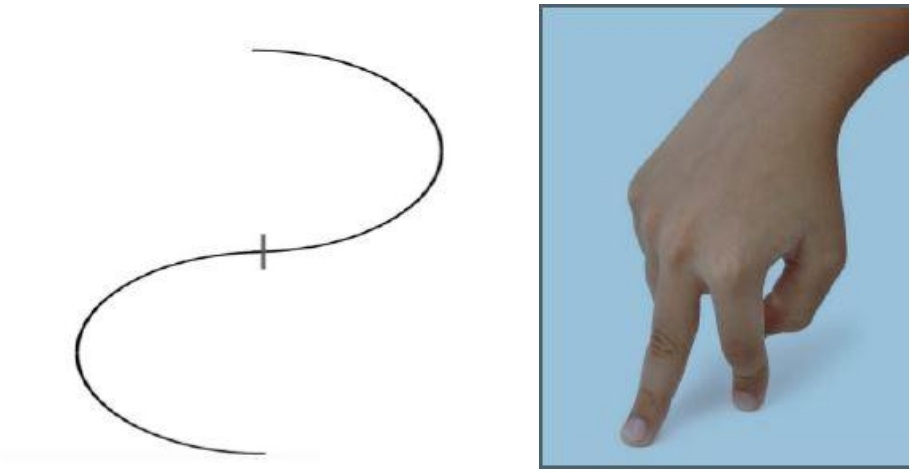


Figure 2.2 The path (left) used for the simulated walk test and the hand movement (right) executed by the subjects (left)

2.4 Data analysis

The main focus of the analysis was to compare the executed and imagined tasks and to explore possible impact of OA in both the conditions. The dependent variables considered was the time needed to execute or to imagine the action, however, previous to be introduced in the analysis, some of the data has been transformed. For both hands and legs simple tasks it has been calculated the time index of a single movement, summing the total amount of time needed for each condition (executed and imagined) and dividing it for the total number of repetitions (5+2+3+4) [130]; the TUG measure has been transformed from time to speed [131] considering the total distance covered during the execution (6m / time needed to complete the task). With the exception of the imagined condition TUG tasks and the single leg movements the data resulted non normally distributed (see table 2.2), for this reason a non parametric approach has been chosen to analyze them.

Two different analysis have been conducted: in the first analysis we compared the performances, imagined and executed, for the tug test, the simulated walk and the single leg and hand movements of our two groups, analyzing separately the left and right limbs for the simple tasks. We obtained a total of 12 Mann Whitney; For the second analysis the data were transformed in six differences, expressed in seconds, obtained subtracting the amount of time needed for executed condition of a task from the time needed during the imagined condition ("imagined – executed"). The values obtained resulted non normally distributed, with the only exception of the executed condition of the TUG test (table 2.3a,b), thus a non parametric approach has been chosen, similarly with the previous analysis. Six different Mann Whitney comparison has been conducted on the absolute values of the differences obtained. The purpose of this second comparison was to compare the level of isochrony of each task between the two groups as an interaction between tasks.

To exclude the possible influence of the educational level on our results test we use a Spearmen test to correlate the years of education with the time, expressed in seconds, needed to perform in each tasks, imagined and executed, for both groups. The same process has been used for the values expressed as differences in the second analysis.

Table 2.3a normality tests for the executed and imagined tasks

		Normality assumption					
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistics	df	Sign.	Statistics	df	Sign.
Tug_executed	PT	0.132	22	.200*	0.95	22	0.318
	HS	0.177	25	0.043	0.861	25	0.003
Tug_imagined	PT	0.13	22	.200*	0.94	22	0.2
	HS	0.113	25	.200*	0.956	25	0.336
left_leg_executed	PT	0.136	22	.200*	0.947	22	0.274
	HS	0.126	25	.200*	0.896	25	0.015
left_leg_imagined	PT	0.137	22	.200*	0.938	22	0.178
	HS	0.114	25	.200*	0.963	25	0.488
right_leg_executed	PT	0.165	22	0.123	0.902	22	0.033
	HS	0.186	25	0.025	0.888	25	0.01
right_leg_imagined	PT	0.169	22	0.1	0.934	22	0.151
	HS	0.098	25	.200*	0.954	25	0.312
simulated_walk_executed	PT	0.175	22	0.078	0.882	22	0.013
	HS	0.196	25	0.014	0.886	25	0.009
simulated_walk_imagined	PT	0.221	22	0.006	0.897	22	0.026
	HS	0.175	25	0.047	0.891	25	0.012
right_hand_executed	PT	0.131	22	.200*	0.94	22	0.195
	HS	0.15	25	0.148	0.908	25	0.028
right_hand_imagined	PT	0.145	22	.200*	0.941	22	0.208
	HS	0.191	25	0.019	0.889	25	0.011
left_hand_executed	PT	0.191	22	0.035	0.889	22	0.018
	HS	0.156	25	0.117	0.926	25	0.069
left_hand_imagined	PT	0.209	22	0.013	0.854	22	0.004
	HS	0.201	25	0.01	0.927	25	0.073

[a] significance correction of Lilliefors

*inferior limit of the effective significance

Table 2.3b Normality test of the six differences, for the two groups

		Normality assumption					
		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistics	df	sign	Statistics	df	sing
tug	PT	0.127	22	.200*	0.945	22	0.245
	HS	0.1	25	.200*	0.95	25	0.252
left_leg	PT	0.127	22	.200*	0.967	22	0.645
	HS	0.148	25	0.162	0.93	25	0.085
right_leg	PT	0.144	22	.200*	0.88	22	0.012
	HS	0.174	25	0.05	0.874	25	0.005
simulated walk	PT	0.176	22	0.075	0.793	22	0
	HS	0.166	25	0.075	0.906	25	0.025
righ_hand	PT	0.192	22	0.034	0.888	22	0.017
	HS	0.256	25	0	0.83	25	0.001
lef_hand	PT	0.182	22	0.056	0.848	22	0.003
	HS	0.187	25	0.025	0.869	25	0.004

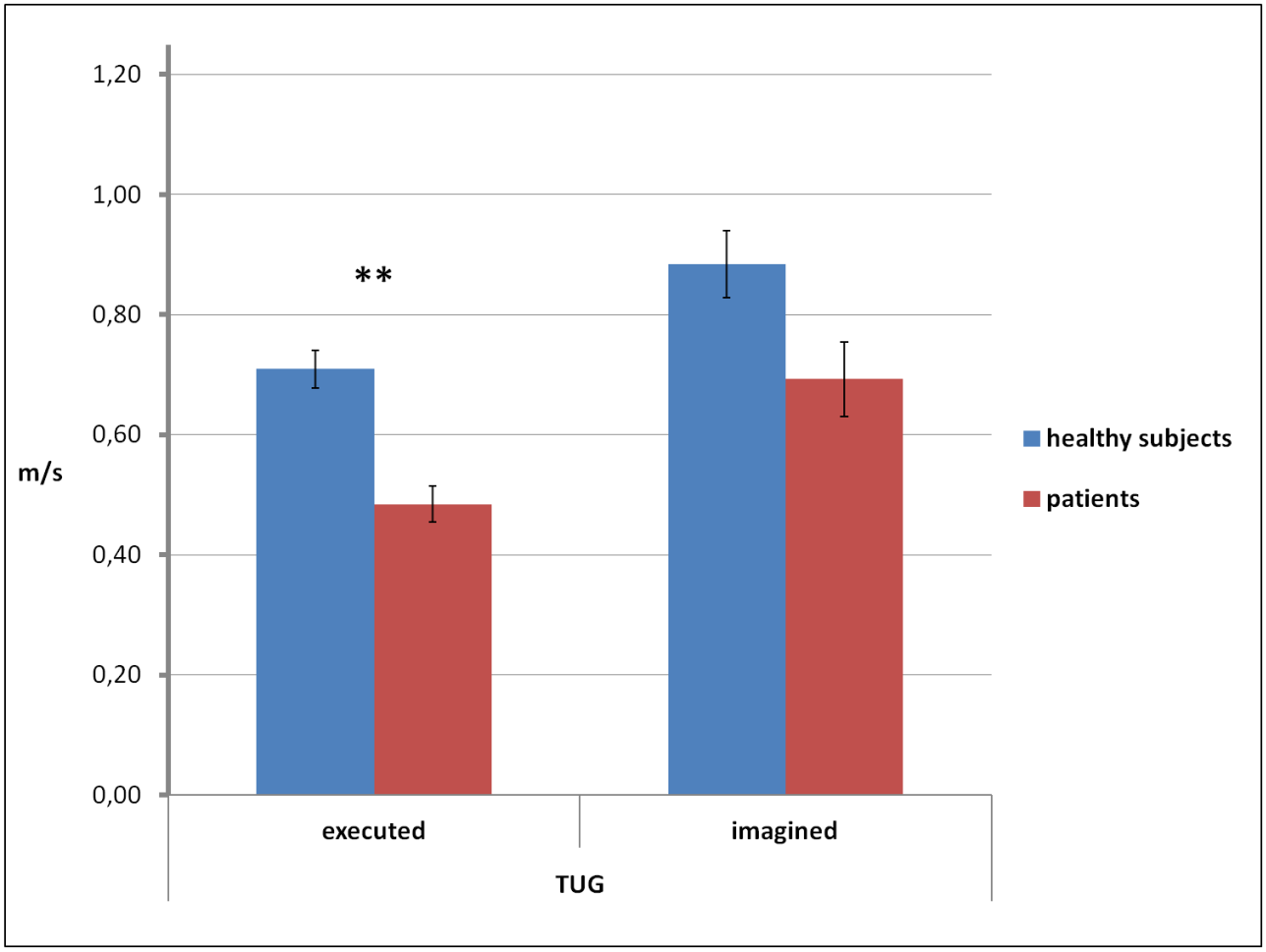
[a] significance correction of Lilliefors

*inferior limit of the effective significance

2.5 Results

In the first analysis a Mann-Whitney has been executed to compare all the 12 tasks, 6 executed and 6 imagined, between the two groups. The significant data that survived the multiple comparison correction **(p≤ .004)** was the TUG executed condition **(U= 68,000; Z= -4413;p= .000)**: the patients **(mean= .49 m/s ,DS= 0.15)** were significantly slower than the healthy subjects **(mean= 0.71 m/s, DS= 0.15)**.The imagined version **(U=171,000; Z=-2217; p=.027)** did not survived to multiple comparison correction, even if a trend similar to execution was reported comparing the two means **(**patients**, mean= 0.69 m/s, DS= 0.26;** healthy subjects**, mean= 0.88, DS= 0.31) (**See figure 2.3).

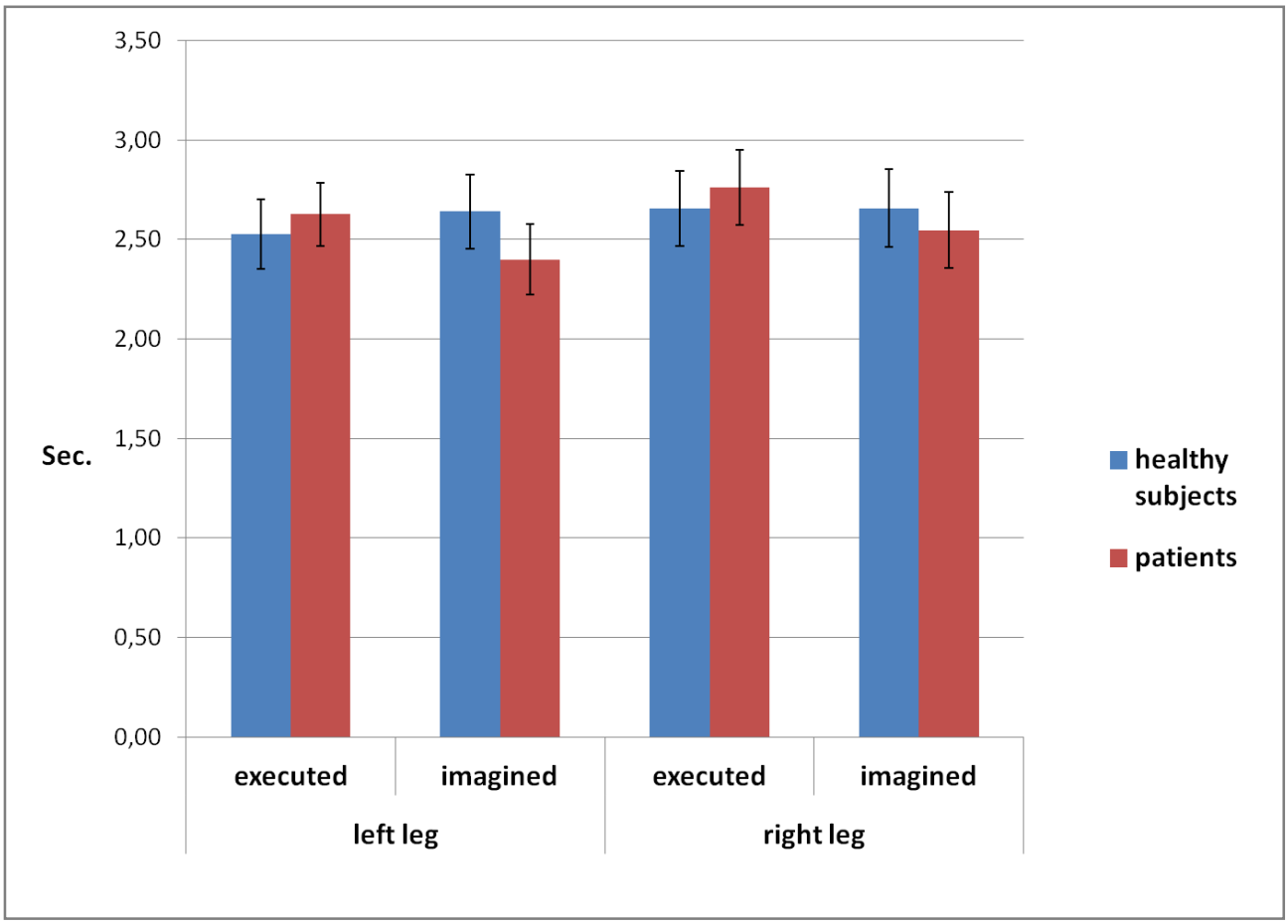
Figure 2.3 TUG test. Speed, expressed in m/s, for performances at the TUG test, executed and imagined, for the two groups. The patients (red) were significantly slower than the healthy controls (blue) in the execution. For each task the standard error mean is also reported.



** significance corrected for multiple comparison (p≤.008)

The comparison for the left of or right leg were not significant in both the executed (Left_leg_executed **(U= 26,000; Z= - 0.32; p=.749)**; Right_leg_executed **(U=26,000 ;Z= - 0.32;p= .749)**) and imagined tasks (Left_leg_imagined **(U= 22,000; Z= -1173; p= .241)**; Right_leg_imagined **(U=252,000 ;Z=-.49;p= .624)**). (see figure 2.4).

Figure 2.4 Simple "leg tasks". Mean of the performances, expressed in seconds, for the executed and imagined conditions for the patients (red) and the healthy subjects (blue). No significant differences emerged between the two groups. For each task the standard error mean is also reported.



No differences were reported between the two groups for the control tasks: the simple hands movements performances were not different in both execution and imagination (Right_hand_executed **(U= 267,000; Z= -.171;p= .865)**; Right_hand_imagined **(U= 244,000 ;Z= -.661;p= .59)**; Left_hand_executed **(U= 23,000; Z= -.959;p=.337)**; Left_hand_imagined **(U= 217,000; Z= -1237;p= .216)**) (figure 2.5).

The same results was obtained for the complex task, the simulated walk task (Simulated_walk_executed **(U= 191.500; Z= -1.78; p= .75)**; Simulated_walk_imagined **(U= 239,000; Z= -.768; p= .443)**) (figure 2.6).

Figura 2.5 Simple hand-movement task. Mean of the performances, expressed in seconds, for the executed and imagined conditions for the patients (red) and the healthy subjects (blue). No significant differences emerged between the two groups. For each task the standard error mean is also reported.

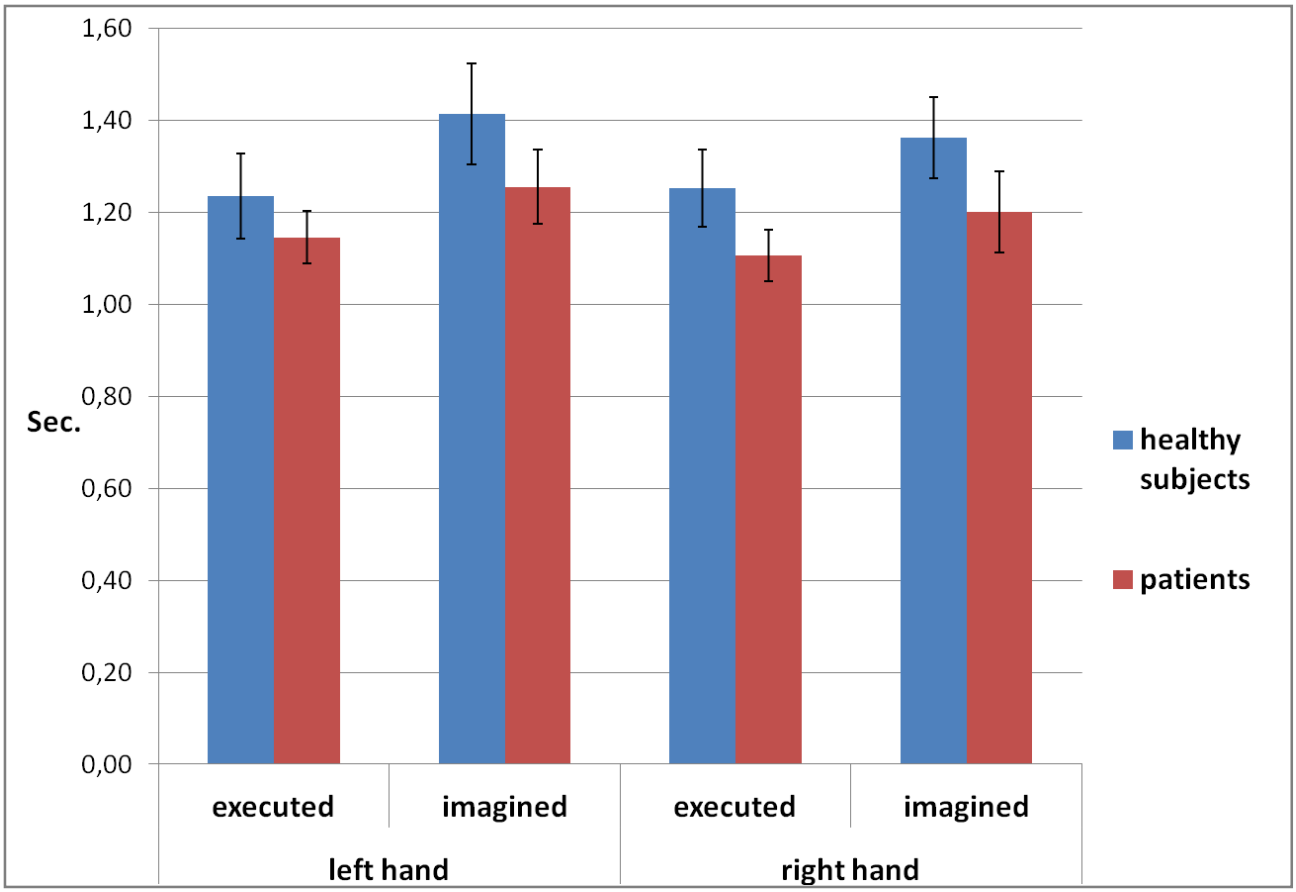
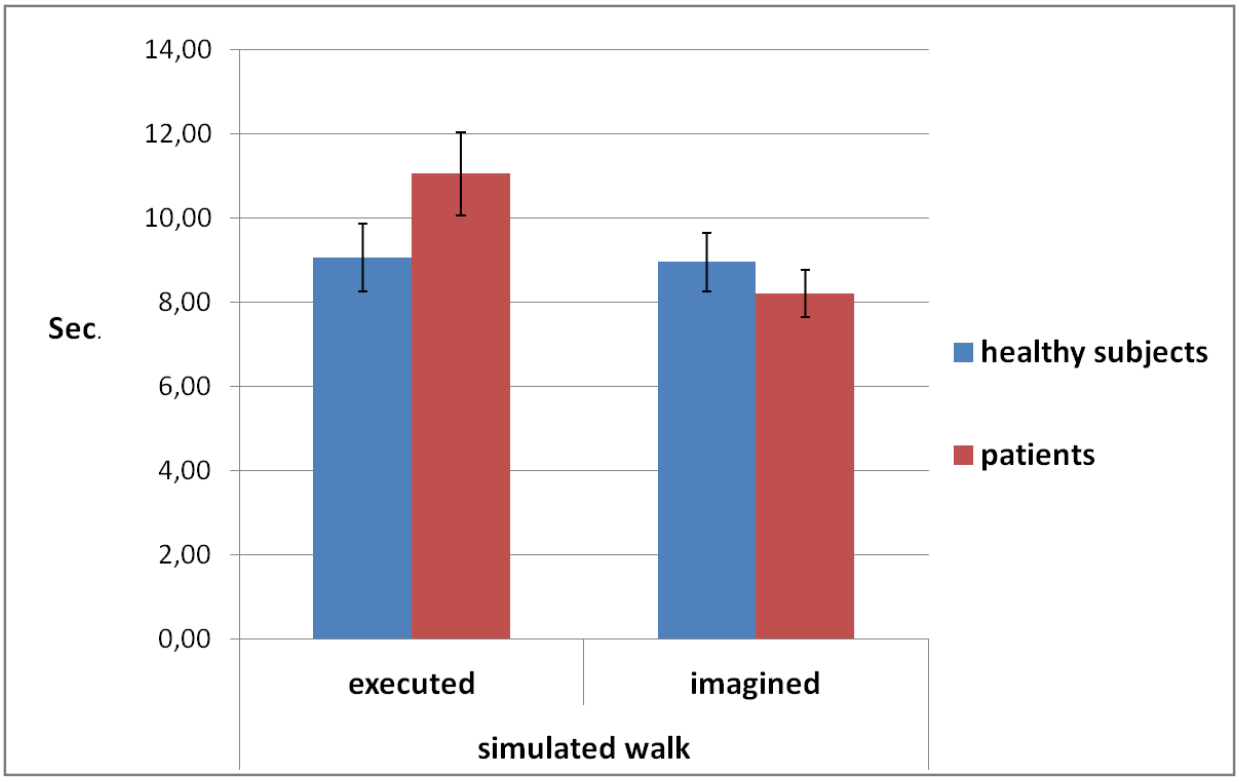
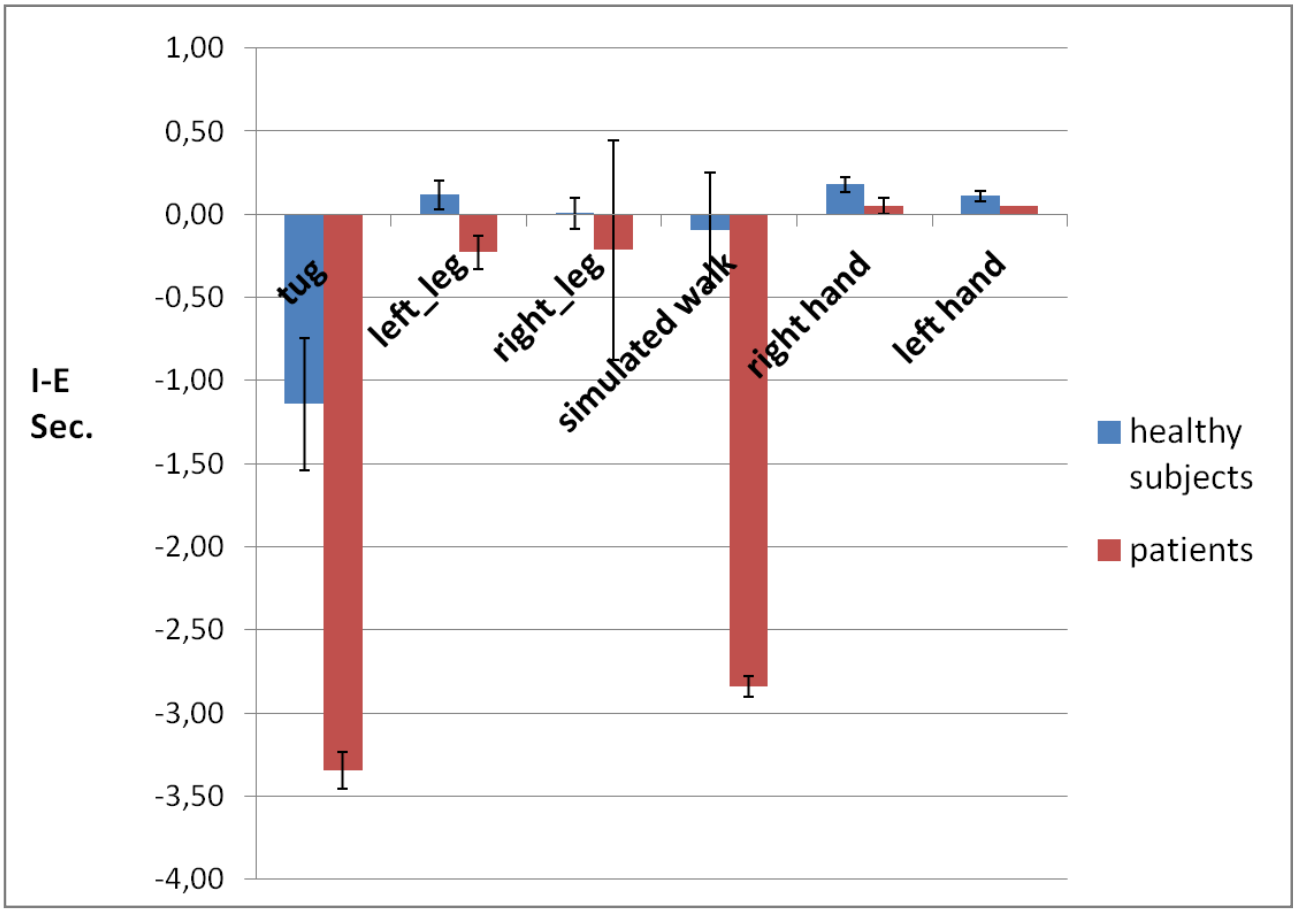


Figure 2.6 Simulated walk task. Mean of the performances, expressed in seconds, for the executed and imagined conditions for the patients (red) and the healthy subjects (blue). No significant differences emerged between the two groups. For each task the standard error mean is also reported.



From the second analysis the level of isochrony, expressed as the difference in seconds of the time for imagination minus the time for execution, did not significantly differ between the two groups for all the tasks, since no difference survived the multiple comparison correction **(p<.008)** (TUG **(U=162,000;Z=-2409;p=.016)**; **left_leg (U=197,500;Z=-1653;p=.098)**; right_leg **(U=262,000; Z= -0.277; p= .782)**; simulated_walk **(U=167,500; Z=-2292; p=.022)**; right_hand **(U=260,500; Z= -0.309; p= .757)**; left_hand **(U=268,500; Z= -0.139; p= .89)**). However, for the TUG there was a substantial trend, mimed also by the performances at the simulated walk task (see fig 2.7).

Figure 2.7 level of isochrony: mean of the differences for each task, expressed as RTs in seconds, rather than speed, for the healthy subjects (blue) and the patients (red). For each task the standard error mean is also reported.



* simulated walk= the stroll along a curvilinear path simulated with two fingers of the right hand

The isochrony was also expressed as the correlations between the performances for the executed and imagined tasks in the two groups. The executed performances were highly correlated with the imagined ones for both groups. (see table 2.8)

Table 2.8 non parametric correlations between the performances executed and imagined in each tasks for both patients (PT) and healthy subjects (HS).

		Tug	Left leg	Right leg	Simulated walk	Right hand	Left hand
PT	p	**.001**	**.000**	**.000**	**.000**	**.000**	**.000**
	ρ	**,657****	**,751****	**,788****	**,720****	**,689****	**,796****
HS	p	**.001**	**.000**	**.000**	**.000**	**.000**	**.000**
	ρ	**,639****	**,818****	**,866****	**,906****	**,861****	**,930****

As previously described we correlated the years of educations of our subjects with the time needed to perform in the tasks, executed and imagined, to exclude the hypothesis that the educational level could influence any identified effect.

The Spearman correlation between the educational level and the performances at the TUG test was not significant for both the patients (execution: **ρ = -.099, p= .662**; imagination: **ρ =.066, p= .771**) and the healthy subjects (execution **ρ= .169** , **p =.419**; imagination: **ρ = .267** , **p= .197**).

2.6 Discussion

For this behavioural experiment I adopted the definition of motor imagery as a form of "mental motor rehearsal" [49, 50], implying a process directly related to the real movement execution: to validate this relation the use of mental chronometry has become one of the privileged methods of investigation. One other method, of course, when it comes to patients populations, is to make comparisons with groups of healthy subjects.

The purpose of my study was to compare the performances for gait related tasks of a group of candidates to total knee arthroplasty as compared to a group of age matched controls: more specifically I was interested to observe whether this pure peripheral pathology would have been able to influence not only the performances for executed task but also for the imagined counterpart.

The benchmarks of preserved motor imagery skills in this context were the comparative speed of execution of the tasks among controls and patients and the comparative coupling of such speeds in the executed and imagined version of the tasks.

These benchmarks are admittedly not simple to interpret.

The so called principle of "functional equivalence", expressed as the isochrony between the amount of time needed to execute an action as compared to the time needed to imagine it, has been used as a parameter to define how good motor imagery ability of an individual is. It should be noted that this principle is not univocal: several studies has reported differences in the stability of the isochrony based on the level of complexity of the task required, leading to a dissociation between the actual execution and its imagined counterpart [110, 111]. The difficulty of a complex tasks could be represented in an underestimation or and overestimation of time during MI: in the first case there's a lack of attention to the details that compose the sequence of a movement; in the second case the attempt to maintain all the elements involved in the action cause a difficulty to sequence them. Also involved in the principle of functional equivalence is the idea of updating: during motor imagery a real executed action is rehearsed, thus any factor or element that could potentially modify the actual performances might be reflected in the imagined version.

This could lead to maintain the isochrony between the executed and imagined condition [132] or create a bias similar to the complexity effect [115] [112].
In my experiment these factors were taken into account.
To test the effect of the gonarthrosis on the motor imagery ability of our patients I created different tasks: besides the TUG test [101], used to measure the gait related performances of our subjects, I added as second measure, defined by a simpler task involving the use of lower limbs. In addition two tasks involving the upper limbs were created, matched for complexity as control tasks: we wanted to be sure to asses that any effect detected for the TUG would have been a consequence of the gonarthrosis and not to be caused by the complexity of the task itself.
Comparisons of the patients' data with the data from controls and within group correlations of the various measures offered two complementary scenarios.

Between-group analysis.

The effect of gonarthrosis revealed by the analysis was an obvious reduced speed for the executed version of the TUG test: the patients were significantly slower than the healthy subjects.
Interestingly, the difference obtained for the executed performances was not replicated in the imagined tasks: it looks as if the patients, on average, imagined as if they were somewhat healthier than they actually were, which suggests that their motor imagery were not updated to their actual clinical condition.
This speculation is supported by the non-parametric analyses on the differences between the RTs for imagined and executed movements (an indirect approach to a non-parametric group by task interaction effect): while this difference was not an overwhelming one (it did not reach a Bonferroni corrected threshold), yet there was a substantial trend in that direction.
Another interesting observation was a substantially larger difference between the imagined form of the "simulated walk" with the right hand and the executed version of the task, an effect only reported for this complex task and not observed in the simpler "fist making" task involving the right hand. This suggests that motor imagery in our subjects is not as efficiently coupled with the motor execution of a task with a weight, due to the complexity of the task. An alternative interpretation of the results obtained for the simulated walk task could be attributed to the nature of the task itself: even if the movement was executed with the hand, the motor program involved in the task might have required the participants to emulate a walking behaviour, thus the same limitation reported for the TUG would have also been reflected in this task.

Within group correlations.

These analyses offer a slightly different perspective because they show that within each group, patients and controls, the correlation between the executed and imagined version of each task were preserved. This further supports the supposition that the patients are actually imagining their movements as if they were healthier than they actually are. In other words it looks as if their motor imagery procedures are following premorbid motoric patterns. The finding generalizes to complex movements with the upper limbs.

To summarize, in this chapter I observed that our patients, besides the obvious reduction of gait speed in the executed movements, had a less realistic motor imagery than normal controls while their motor imagery skills were still available.

In other words, their functional impairment was not severe enough to compromise in a dramatic manner the imagined counterpart of the gait related task.

However, the qualitative differences between healthy controls and patients could be also due to a strategic shift of the gonarthrosis patients away from a imagery activity that is motoric in nature. It is not readily possible to test this hypothesis on the basis of our behavioural tests. However, the fMRI experiment described in chapter 4 may help in deciding among different possibilities when trying to interpret the behavioural data.

CHAPTER 3 - *The functional brain correlates of motor imagery and imitation of gait An fMRI: investigation on healthy subjects*.

In this chapter, I will present an fMRI study conducted to identify the network involved in locomotion control as measured by gait-related tasks using the so-called "virtual walking stimuli" (see materials and methods paragraph). The same paradigm was used to compare the networks involved in a motor imagery (MI) and an action observation (AO) condition: in the MI task, defined as "imagery", the subjects were asked to imagine walking along a series of visually presented naturalistic moving scenarios; in the (AO) condition, defined as "imitation", the subjects were asked to imagine imitating the action of an actor filmed from behind while walking along the same naturalistic scenarios. In addition, in both the imagery and imitation conditions, the participants were asked to execute an ankle dorsiflexion concomitantly to presentations of half of the stimuli. After a brief introduction on locomotion control literature regarding the MI and AO for gait related tasks, I will describe the materials and methods of the experiment and the performed analysis, and finally discuss the results obtained.

3.1 Introduction

Gait networks

The gait's double nature encompassing both highly automatized behaviour and complex motor programming is also represented at a neural level, involving both spinal and supraspinal structures.

At a spinal level, there are the so-called *"central pattern generators"* (CPGs) [133, 134], which can produce the stereotyped patterns of muscle contraction involved in locomotion. It has been demonstrated that CPGs can fulfil their purpose in complete independence from the higher level structures, as observed in patients with spinal transection [135-137] and complete paraplegia [135, 138].

Moving up from the spinal cord, a further distinction is needed between sub-cortical and cortical regions. Information on the subcortical organization for gait modulation mainly comes from animal models. The subcortical regions are organized in a network which is specific for locomotion and includes the *mesencephalic locomotor region* (MLR), that projects to the *subthalamic locomotor region* (SLR), located in the reticular formation in its ponto-medullary part, and the *cerebellar locomotor region* (CLR) [11, 139, 140] (see Chapter 1). The subcortical network is responsible for gait initiation, stepping modulation and on-line adaptation to the environment, defining speed, force

and velocity. In particular, the MLR has been defined as the "pacemaker" of locomotion [10] and is capable of regulating its rhythm.

The human counterpart of the animal subcortical network has been observed in the pedunculopontine region and the subthalamic nuclei, as observed by Jahn and colleagues [141, 142] using fMRI and MI tasks (see Figure 3.1).

At a cortical level, several areas are involved in adapting the gait behaviour to the different environmental influences and contexts through descending modulation of the subcortical networks, as described for the *higher level gait disorders* [19] (see Chapter 1): together with the M1 and S1 cortices, the PM and SMA showed to be involved in locomotion adaptation, as observed in the clinical context for patients with "gait apraxia" and other deficits that involved gait disturbances with spared motor capacity [16-18] .

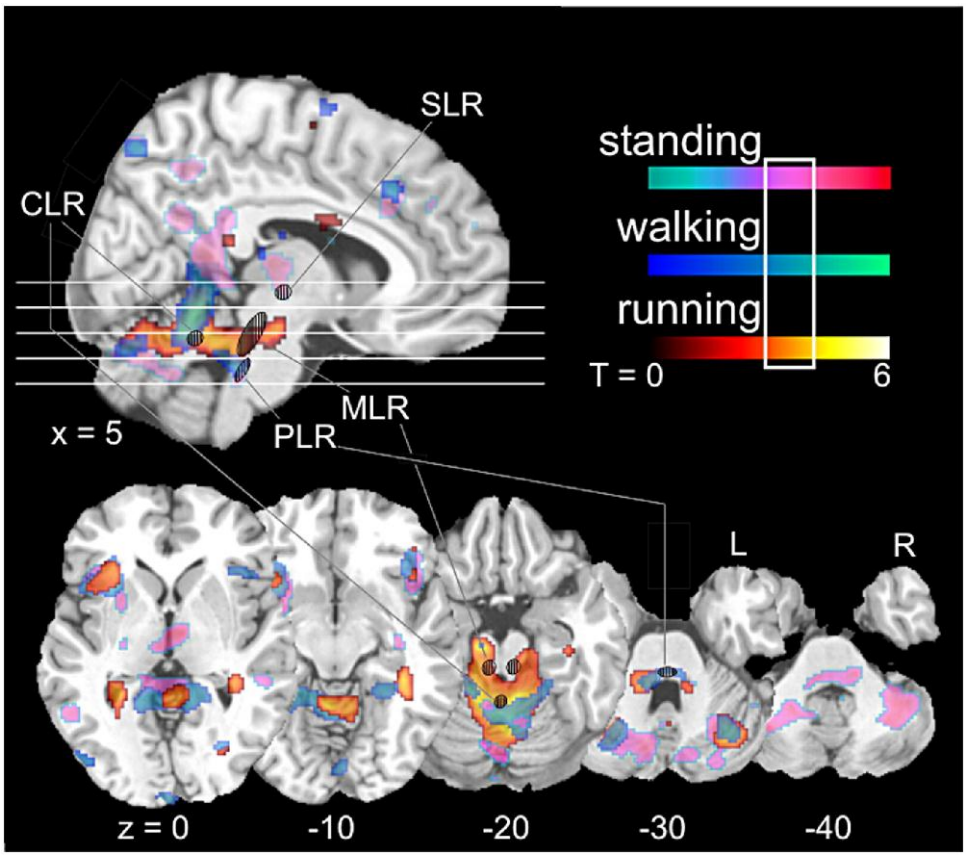


Figure 3.1 BOLD signal changes between standing, walking, and running ($p < 0.05$, FDR). The network involved a large cerebellar and midbrain activation during running, partially overlapped during walking, and the activations in the thalamus, precuneus, and caudal pons during standing. The black circles and ovals indicate what is considered the human homologues for the regions in the cat. CLR—cerebellar locomotor region (fastigial nucleus in midline cerebellum); MLR—mesencephalic locomotor region (corresponding to pedunculopontine and cuneiform nuclei in the mesencephalic tegmentum at the level of the inferior colliculus); PLR—pontine locomotor region (pontine reticular formation); SLR—subthalamic locomotor region (subthalamic nucleus); L—left; R—right. (Jahn et al. 2008 page 789)

Other proofs of the cortical influences on gait ability have been reported for white matter lesions [143, 144] and whole brain and gray matter reduction [145].

fMRI study for gait-related tasks in healthy elderly

Due to its complexity, gait is indeed cognitively demanding and defining the impact of aging on locomotion control has become more and more important: a direct relation between brain and the physical performances along the life course has been reported with specific regard to the influence of the global volume of grey and white matter and the quality of gait, expressed as speed and strength. [145] The global cognitive functions, and especially the executive functions, are considered to have a large influence in defining gait speed decline [146] and also have an important role in the prevention of falls [147]. For this reason, fMRI could be a useful tool to define the neural networks involved in gait control in healthy elderly and add new information on normal aging.

The fMRI environment heavily reduces the possibility to test cortical activation during the execution of real locomotion: to overcome this problem, alternative strategies have been proposed (see Chapter 1), including the use of limited movements, such as ankle dorsiflexion [44] or the use of MI and AO. Zwergal et al. [148] have recently used MI to acquire information on possible compensatory processes, as observed for other tasks [130, 149-151], for gait control in healthy elderly. Their results showed the involvement of multisensory vestibular, motion sensitive (V5/MT) and somatosensory (right postcentral gyrus) areas for gait control in the MI task with preserved activation of SMA and the subcortical networks. These results are in line with the hypothesis of a shift between a less automatic and more attentional behaviour with aging.

Other studies that have reported activation in both cortical and subcortical networks using explicit gait-related MI tasks are those of Jahn and colleagues, as previously anticipated [141, 142] (fig 3.1). A study with a different approach was proposed by La Fougère [43], who combined the use of MI tasks in fMRI with the activation for real locomotion using 18FDG PET, reporting a shared cortico-subcortical network: the activations are reported in a network involving the frontal cortex, the cerebellum, the pontomesencephalic tegmentum, the parahippocampal, fusiform and occipital gyri, in association with the deactivation in the multisensory vestibular cortices.

As previously explained, another method for gait study in the fMRI context is AO as proposed by Iseki et al. [97]: the authors compared the passive observation of an actor walking in a third person perspective with the observation of a moving scenario in a first person perspective, constructed to elicit the mental imagery of gait. The authors reported the activation of cortical areas such as SMA and PM cortex in both situations, with the involvement of more posterior areas probably due to the elaboration of the visuo-spatial information contained in the presented stimuli. In their experiment, the involvement of subcortical networks was not clear. The absence of subcortical activation could

be explained by the instruction used by the authors: the subjects were not required to explicitly imagine executing the action. On the contrary, in their studies Wang and colleagues [152, 153] asked the participants to actively imagine executing the movement while watching the leg of a model walking from a first-person point of view: the activation network of activation reported included the SMA and PM with the associated recruitment of the inferior and middle frontal regions, the parietal regions, occipital areas including MT/V5 and also the cerebellum. Dalla Volta and colleagues [98] asked participants to execute a step-like movement on a rolling cylinder, positioned under the participants' feet during the fMRI scan, and passively watching the same action executed by an actor: they found a neuro-functional overlap between the observation and execution tasks in SMA and PM, superior and inferior parietal areas and the cerebellum, but again no brainstem activation.

In summary, the activation at brainstem level that has been reported for gait imagery seems to be missing in gait action observation: however, it is important to underline that the two tasks have never been compared in a unitary procedure, with matched visual stimuli and instructions. Particularly in the elderly, the importance of controlling the visual stimulation becomes extremely relevant: previous studies have reported how gait control becomes more attention demanding and depending on environmental cues with aging [154] [155] and how this reflects on the ability to mentally evoke the motor representation of walking [148].

3.2 Aim of the study

The purpose of this study was to directly compare the effect a condition of MI for gait related, defined imagery, with tasks that associated MI & AO for matched stimuli. For our purpose, we created an fMRI paradigm using a set of virtual walking stimuli [97, 156-158], i.e. walking on a path in a park shown from a first person perspective, in order to evoke MI more easily. The participants were asked to imagine walking along the paths shown, as if the camera was their own eyes. To test the effect of AO, a set of visually matched stimuli was created, with the addition of an actor/actress filmed from behind while walking along the same path: differently from Iseki et al. [97], the subjects were asked to actively imagine imitating the action of the actor; moreover, the perspective of the actor was compatible with the observer to allow the possibility of "shadowing" the movement without the spatial remapping involved in the observation of an actor presented frontally, and facilitating the recruitment of motor processes during the tasks [159, 160]. For each version, a baseline was created presenting a still scenario, with (AO) or without (MI) an actor

standing still: in this study condition, the participants were asked to imagine staying still or imitating the actor's movement. Finally, another change was introduced: in 50% of the cases, the presentation of the visual stimuli was associated with a rhythmic ankle dorsiflexion [44], mimicking the stepping rhythm, for the experimental and baseline condition in the AO and MI tasks. To summarize, this study through the use of the virtual walking task had three purposes: to describe the neural network involved in locomotion control for motor imagery, to test possible modulatory effects of action observation, and to define their relation to the motor network involved during the real ankle dorsiflexion, a fMRI-compatible proxy for motor performance during walking.

3.3 Materials and methods

Participants and neuropsychological assessment

24 healthy elderly participants took part in the study (10 f, age 66.79 ± 6.97, education 13.54 ± 4.14). All participants were right-handed as confirmed by the Edinburgh Handedness Inventory [126], and reported normal or corrected-to-normal vision. The experimental protocol was approved by the Local Ethics Committee (Comitato Etico Azienda Sanitaria Locale Città di Milano), and was carried out in accordance with the ethical standards of the 1964 Declaration of Helsinki. All participants were without medical history of neurological or psychiatric disorders and provided their informed written consent to take part in the fMRI study. To exclude age-related cognitive deficits, all participants also completed the Mini-Mental State Examination [127], a test providing a summary index of cognitive functioning: it proved that no participant showed signs of cognitive impairment (all scores > 24), confirmed by the Raven's Coloured Progressive Matrices [128]. Finally, participants also completed the Vividness of Movement Imagination Questionnaire (VIMQ) [129], a self-report questionnaire on explicit motor imagery abilities (mean scores in motor imagery/first person = 46.04 ±16.44, visual imagery/third person = 44.71 ±16.41, indicating good self-reported motor imagery abilities). (table 3.1)

Table 3.1 Demographic and neuropsychological data of the participants

N		Age	Education (years)	MMSE (Corrected)	Raven (Corrected)	VMIQ 1st	VMIQ 3rd
24(F=10)	mean	66.79	13.54	29.09	33.32	46.04	44.71
	SD	6.97	4.14	1.51	3.48	16.44	16.41

fMRI paradigm: stimuli and tasks.

The experimental paradigm was divided in two runs to separately study AO and MI: I will refer to the MI task as "imagery", while "imitation" defines the AO task. The two paradigms were constructed with the same structure. The subjects had to imagine walking ("virtual walking") or standing still ("standing") while watching short movies representing still or moving scenarios filmed from a first-person perspective, trying to imaging that the camera was their own eyes.

The structure was composed of these elements:

- The entire runs were composed of 230 3-second scans and organized in a block design structure.
- The first 30 seconds only contained the instructions of the task.
- The stimuli were composed of single short movies, 15 seconds each, representing a naturalistic path with different slopes (down-hill or low-hill) filmed from a first-person perspective. 8 different versions of the stimuli were created for each task.
- The visual stimuli were presented in pairs: each time, the same scenario was presented in the still condition, followed by the moving condition.
- Every stimulus was preceded by an instruction asking the subjects to stay still ("no feet movement") while the video was running, or to associate the dorsiflexion of the feet to the presentation ("move your feet").
- Eight times per run, a question followed the stimuli asking the subjects to judge whether the slope previously observed was uphill or downhill.

Starting from this common structure, two versions were created.

The stimuli were created especially to make the subject feel more immersed in the virtual environment, gaining more details of the imagined fatigue experienced to perform the task, thanks to texture variation of the paths, with the aim of giving subjects a more realistic experience of motion perception with the use of the moving scenarios.

Adding the moving scenarios was a means to further support the attempt of the subjects to imagine a realistic rhythm of locomotion, while the dorsiflexion was introduced to compare a real movement, consistent with locomotion, while being involved in the imagery task.

The instruction in the "imagery" (MI) task, previously explained to the subjects, was to imagine walking or standing still while observing the scenario alone, trying to synchronize the imaginary steps to the video speed. While imagining, the subjects had to focus especially on the physical and kinesthetic sensations associated with the movement involved in gait; the task was therefore more centered on kinesthetic MI than visual MI, even if associated with a visual presentation. For half of the stimuli, both the moving and the still scenarios, the subjects were asked to move their ankles,

executing an alternated dorsiflexion, while watching. The movement was associated both in the moving and the still condition.

In the "imitation" (AO) task the instruction was to imagine imitating an actor (2 men/2 women, alternating), walking or standing still, along the same scenarios presented in the imagery task. The subjects had to imagine executing the same movement, trying to imagine the sensations experienced by the actor based on the action observed and the muscle involvement needed by the actor to adapt to the different slopes. As for the imagery tasks, the subject had to associate the alternated ankle dorsiflexion to half of the stimuli presented. In this second task, the subject used the speed of the actor to set the speed of the imagined steps. The actors were filmed from their back, so that the observer could imagine walking behind the actor, shadowing his/her movement. (Figure 3.2)

figure 3.2 Example of the fMRI stimuli for the imagery (left) and imitation (right) conditions.

Stimuli presentation was controlled by Cogent 2000 MATLAB Toolbox (Math Works, Natick, Ma, Usa). Visual stimuli were delivered using Visuastim fibre-optic goggles (600 x 800 pixel resolution). Responses were recorded through a response box placed under the right hand (Resonance Technology Inc.).

3.4 Data acquisition and analysis

Data acquisition.

MRI scans were performed using a 1.5 T Siemens Avanto scanner, equipped with gradient-echo echo-planar imaging (flip angle 90°, TE = 60 ms, TR = 3000 ms, FOV = 280 × 210 mm and matrix = 96 × 64). Slice thickness was 5 mm.

We collected 230 volumes for each fMRI run. The first two brain images (TR periods) from each functional run were removed to allow for steady-state tissue magnetization, and additional 10 volumes (corresponding to the task instructions) were discarded from the analyses.
MPRAGE High-resolution T1-weighted structural images were also acquired (160 slices, flip angle 35°, TE = 5 ms, TR = 21 ms, FOV = 256 x 192 mm, matrix = 25 × 192).

Preprocessing.

After image reconstruction, raw data visualization and conversion from the DICOM to the NIfTI format were performed with MRIcron (www.mricro.com) software. All subsequent data analyses were performed in MATLAB R2014b (Math Works, Natick, Ma, Usa), using the software statistical Parametric Mapping (SPM12, Wellcome Department of Imaging Neuroscience, London, UK).
To improve the signal-to-noise ratio, the scans of each subjects' run underwent different passages:
- Re-alignment: all the unwarped functional images of the entire run were re-oriented to the first scan acquired to define a mean scan to reduce the possible artifacts due to motion.
- Co-registration: Co-registration is a process that allows to re-orient the mean functional realigned image obtained from the realignment on the structural T1 reconstructed image of the same subject. Before use, the T1 is segmented to isolate the white matter, the grey matter, the air, the bone and the cerebrospinal fluid, since we were only interested in matching the functional activations with the grey matter distribution.
- Normalization: the co-registered images are then stereotactically normalized into the SPM12 template (tmp.nii) to allow for group analyses of the data [161]. Then, deformation fields used for T1 segmentation were also applied to the unwarped functional scans. At this stage, the data matrix was interpolated to produce 2 × 2 × 2 mm voxels.
- Smoothing: the anatomically normalized images are transformed into probabilistic maps to increase signal-to-noise ratio. The process implies the application of a 10 × 10 × 10 mm Gaussian filter and the extraction of the higher probability of belonging to a determined area for all the voxels contained in the kernel. This process reduces spatial resolution but increase the reliability of the extracted voxels.
- Artifact detection Tools: ART (Whitfield- Gabrieli, http://www.nitrc.org/projects/artifact_detect) allowed to exclude scans, considered as outliers, in global signal and movement for each participant. A difference of over three standard deviations of the mean for scan-to-scan global signal was used as a threshold to mark the timepoints as outliers, while for the scan-to-scan movement the threshold was defined by a compounded measure parameter exceeding 1 mm (on average, excluded volumes were 13.52 ± 8.47 and 9.14 ± 8.41 in the imagery and imitation run

respectively, equal to 6.15% and 4.15% of total scans). 20% of outlier timepoints in a run was considered the limit to maintain a subject in the analyses: two participants were excluded according to this criterion and one participant because of artifacts in the signal, resulting in a final sample of 21 participants.

Statistical analyses of the fMRI data.

A statistical analysis in two steps was then performed, based on the general linear model (GLM) [162, 163]. First, the blood oxygen level-dependent (BOLD) signal associated with each experimental condition was analyzed by a convolution with a canonical hemodynamic response function [164]. The signal was cleaned from any possible fMRI signal artifacts due to vegetative activity, such as noise from cardiac and respiratory cycles, were removed using a High-pass filter (128 s). In the GLM, the realignment parameters calculated in the preprocessing step were inserted as regressors of no interest.

This initial step was a fixed-effect analysis in which condition-specific effects were calculated.

Two aspects have been used to define the conditions:

- The imagined tasks; i.e. imagery of standing (Stand) and imagery of walking (Walk);
- The real movement execution; defined as presence (Mov+) or absence (Mov-) of the ankle dorsiflexion.
- The results were for 4 conditions: two associated with the ankle dorsiflexion, Stand (Mov+) Walk(Mov+), and two with no movement involved, Stand(Mov-) and Walk(Mov-)

The scans corresponding to the 3 seconds of written instruction image ("resting feet" or "move your feet"), the presentation of the alerting questions about the videos and the time-window to record participants' response (8 s in total) were considered as regressors of no interest in the analysis.

Two orthogonal first-level fixed-effect analyses were performed for each fMRI session of each participant. The main effect of the ankle dorsiflexion (feet movement analysis) and the comparison of AO versus MI (gait imagery analysis) were analyzed separately, creating two different sets of contrast images which were inserted into two separate second-level full-factorial ANOVAs that conformed to random effect analyses [162, 165, 166] .

Gait imagery analysis

This analysis aimed at investigating the neural correlates of the motor imagery of gait during virtual walking, with or without the association of action observation (imagery vs. imitation). To isolate the

activations due to AO or MI, the condition of standing still was used as a baseline and subtracted from the walking condition, in order to remove the activation due to the visual observation of the scenario. Contrast images containing voxel by voxel information of the effect of imitation and imagery were created (Walk (Mov-) minus Stand (Mov-); Walk(Mov-) minus Stand (Mov-) for the two runs separately.

We obtained two series of linear contrast:
Effect of Gait Imagery or Imitation when no feet movements were required **(Mov-)**

1. Walk (Mov-) > Stand (Mov-) in the imagery run
2. Walk (Mov-) > Stand (Mov-) in the imitation run

Effect of Gait Imagery or Imitation when no feet movements were required but cancelled out in the contrast **(Mov+)**

1. Walk (Mov+) > Stand (Mov+) in the imagery run
2. Walk (Mov+) > Stand (Mov+) in the imitation run

Importantly, in the latter contrasts, the effect of lower limb movements was cancelled out, because it was present both in the experimental conditions (Walk) and in their respective baselines (Stand), and so it was subtracted by creating the contrast images: for this reason, the contrast 'Walk(Mov+) > Stand(Mov+)' was defined as 'visual', because it identifies the activations related to the visual processes of in-motion stimuli applied in the Virtual Walking tasks, without the motoric component. On the other hand, the contrast 'Walk(Mov-)' > 'Stand(Mov-)' was defined as a visual&motor effect, because it represents the combined visual and (implicit) motor processes involved in the virtual walking imagery, since the possible noise due to the visual information was cancelled by the use of the baseline, as for the movement execution noise (see Figure 3.3 a, b).
Contrasts were thus entered into a second-level full-factorial ANOVAs having Task (Imagery vs Imitation) components (visual&motor vs visual alone), and as within-subject factors, and the effects of interest were further tested by calculating the following linear contrasts to generate SPM[t] maps:

- Main effect of the motor component of virtual walking (visual&motor > visual), contrast 1 1 -1 -1;
- Motor component of Imagery: Imagery(visual&motor) > Imagery(visual alone), contrast 1 0 -1 0;

- Motor component of Imitation: Imitation(visual&motor) > Imitation(visual alone), contrast 0 1 0 -1;
- Conjunction analysis reporting gait-related motor activations in the Imagery and Imitation run, which establishes the neural correlates common to Imagery and Imitation, i.e. voxels significantly activated in both effects (contrasts 1 0 -1 0 ∩0 1 0 -1, Imagery (visual&motor) > Imagery (visual) ∩ Imitation (visual&motor) > Imitation (visual));
- Interaction effects reporting the motor component (motor&visual > visual) in the Imagery > Imitation task (1 -1 -1 1) and in the Imitation > Imagery task (-1 1 1 -1).

All analyses were conducted at the whole-brain level, and thresholded at $p < .001_{uncorr}$. Only peaks in significant clusters are reported. Voxels that survived the FWE correction are also reported. The regional effects that also survived a voxel-wise and cluster-wise FWE correction are reported in the Tables. The main effect of the motor component of virtual walking, together with the motor component for both imagery and imitation, were masked by the respective simple effects of each condition (thresholded at p < .001) to only include voxels which were significantly activated during the conditions of interest.

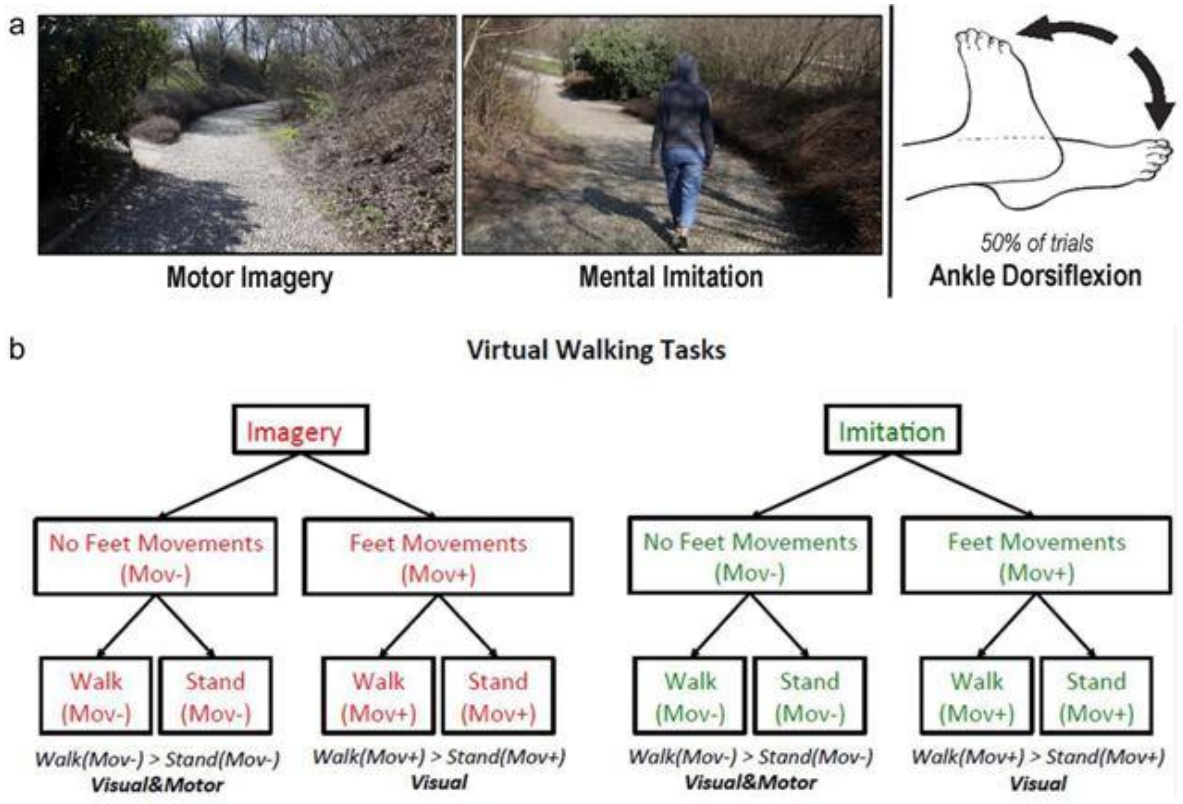


Figure 3.3 A) Example of the stimuli used in the imagery (MI, left) and imitation (AO, right) condition with a graphic depiction of the ankle dorsiflexion and its percentage of occurrence. B) The contrast obtained by the full factorial analysis; Mov+/- represent the presence or absence of feet dorsiflexion; walk and stand are referred to the stationary or moving versions of the visual stimuli.

Feet movement analysis.

In this analysis, the network involved in the ankle dorsiflexion was defined. The other aspect considered was the possible interaction between the explicit movement and the gait imagery and gait observation. To extract this information, the condition of "walking" and "standing still" were compared.

The contrast images allowing such evaluation were derived from the following linear contrasts, which were entered in the second level analysis:

Effect of Feet Movement during Imagery or Imitation of Standing still **(stand)**

1. 'Stand(Mov+) > Stand(Mov-) in the imagery run,
2. Stand(Mov+) > (StandMov-) in the imitation run

Effect of Feet Movement during Imagery or Imitation of Walking **(walk)**

1. Walk(Mov+) > Walk(Mov-) in the imagery run,
2. Walk(Mov+) > Walk(Mov-) in the imitation run.

A full factorial repeated-measure ANOVA was performed having two within-subject factors: Action (Walk/Stand) and Task (Imagery/Imitation). The first step was to define the linear contrasts to generate SPM[t] maps with the intent to identify the overall *effect of feet movements* (linear contrast +1 +1 +1 +1) compatible with the gait behaviour network. This procedure allows to map out the regions involved in feet movement control per se, controlling for the impact of active imagery or imitation of walking. We were specifically interested in testing whether the execution of the ankle dorsiflexion, representing a movement compatible with walking behaviours [44], would facilitate the recruitment of neural resources associated with gait motor control during our tasks. The results have been used to compare the network involved in the feet movement in the imitation and imagery analysis. We also tested the main effects and interactions of the second-level ANOVA to assess whether motor imagery and imitation of walking have a modulatory effect on the neural correlates of explicit feet movements.

3.5 Results

Gait imagery

Motor components of virtual walking

The activations for the "virtual walking task" (expressed as the overall main effect of the task, 1 1 1 1 contrast) are reported in table 3.2a,b APPENDIX A (see Figure 3.4a). The task recruited a large posterior network comprehending cortical areas such as the inferior parietal gyrus and precuneus, the superior and middle occipital gyri, and the superior temporal gyrus together with the right inferior frontal orbital gyrus. In addition to the cortical areas, the thalamus, the putamen and the cerebellum are also activated, as expected in view of the complexity of the virtual walking stimuli and in accordance with the subcortical involvement of gait related areas (see Chapter 1).

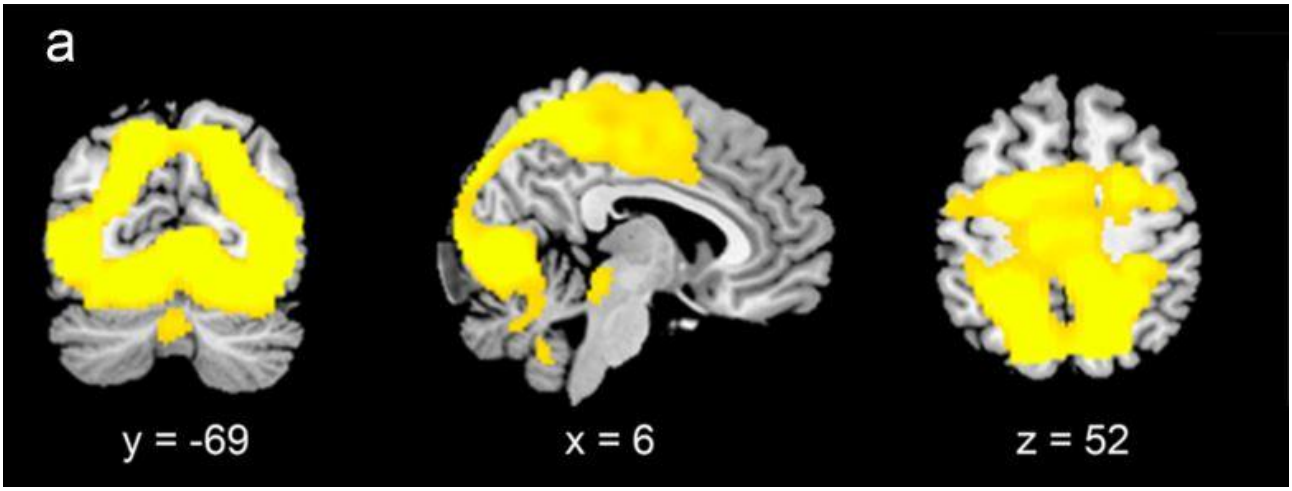


Figure 3.4a Overall main effect for the virtual walking task

More motor related areas appeared in the same network after cancelling out the visual information (visual&motor > visual): activations have been reported in the bilateral fronto-temporo-parietal and cerebellar network including inferior, superior and middle frontal cortices, the SMA, the superior parietal and supramarginal gyri, and the superior and middle temporal gyri (mainly on the left). White matter corresponding to the cerebellar pedunculus and cortico-ponto-cerebellar fibers also appear to be activated in two clusters (see Figure 3.4b) (Table 3.3 APPENDIX A). Figure 3.4b also shows the overlap between the actual movement execution and the virtual walking task.

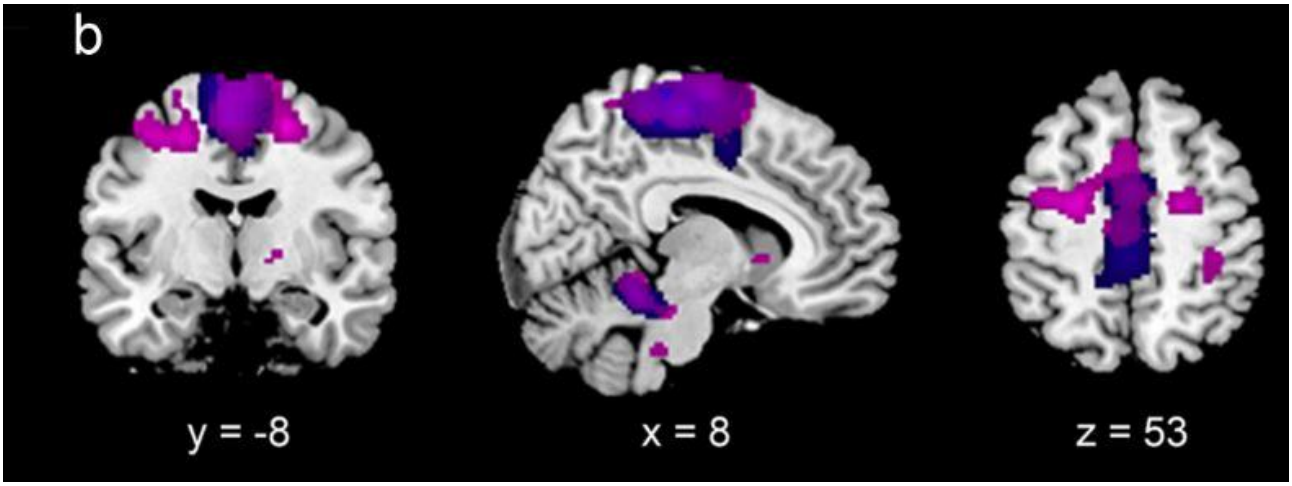


Figure 3.4b Activations for the visual&motor > visual contrast (purple) overlapped with the activations reported for ankle dorsiflexion (blue)

No significant activations have been reported for the opposite analysis (visual > visual&motor) for $p < .001$ uncorrected even without cluster restriction. These data are congruent with the feet movement analysis (see below), which showed how feet movement did not interact with the imagery task (no difference between imagining standing or walking for the feet movement activations), and confirm that the "Mov+ "condition could be used as a control condition for the "Mov-"

Motor components of virtual walking during imagery and imitation

After identifying the overall main effect for the gait-related motor activation during virtual walking, the (visual&motor>visual) two conditions (imagery and imitation) have been considered separately (see Figure 3.5). The imagery condition recruited a network that involved the right orbitofrontal cortex and bilaterally the insula, the middle frontal gyrus, the SMA and the paracentral lobule. Significant activations have also been found in the rolandic operculum, superior parietal gyrus, superior temporal pole, thalamus, and pallidum. The contrast also showed activation in the vermal and paravermal cerebellum extending to the midbrain via the superior peduncule (Table 3.3a APPENDIX A). The imitation condition was associated with significant BOLD signal changes in a smaller network that included the right precentral gyrus, the paracentral lobule, the cerebellar vermis and the left anterior cerebellar lobe (Table 3.3b APPENDIX A). When compared by conjunction analysis, the two tasks revealed common activation only in the cerebellum (Table 3.3c APPENDIX A) .

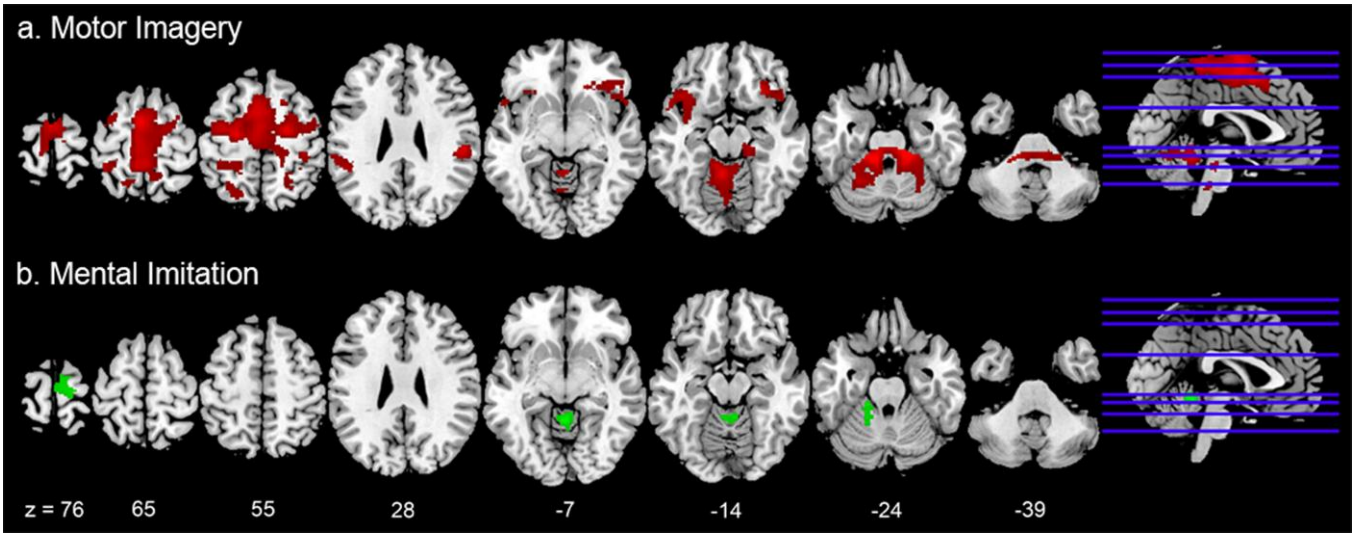


Figure 3.5 Gait-related motor activations for the imagery (red) and for the imitation (green) conditions

Interaction effect between the imagery and imitation conditions

The calculation of interaction effect between tasks (imagery versus imitation) and components (visual&motor vs visual) had the purpose to describe the possible modulation on the motor gait-related networks during the "virtual walking" task due to the presence of a walking actor.

For the imagery condition, i.e. without the actor, the results showed a higher activation in the right parietal cluster, from the parietal operculum to the supramarginal gyrus, in comparison to the imitation condition. In addition, a small cluster of activation was reported in the left exterior cerebellum and in a part of the brainstem compatible with the Mesencephalic Locomotor Region (MLR) (see Chapter 1 and the introduction of this chapter; Table 3.4a, b; Figure 3.6). The reverse interaction, i.e. motor component (Visual&Motor > Visual) x Task (Imitation > Imagery) effect, did not show any significant results.

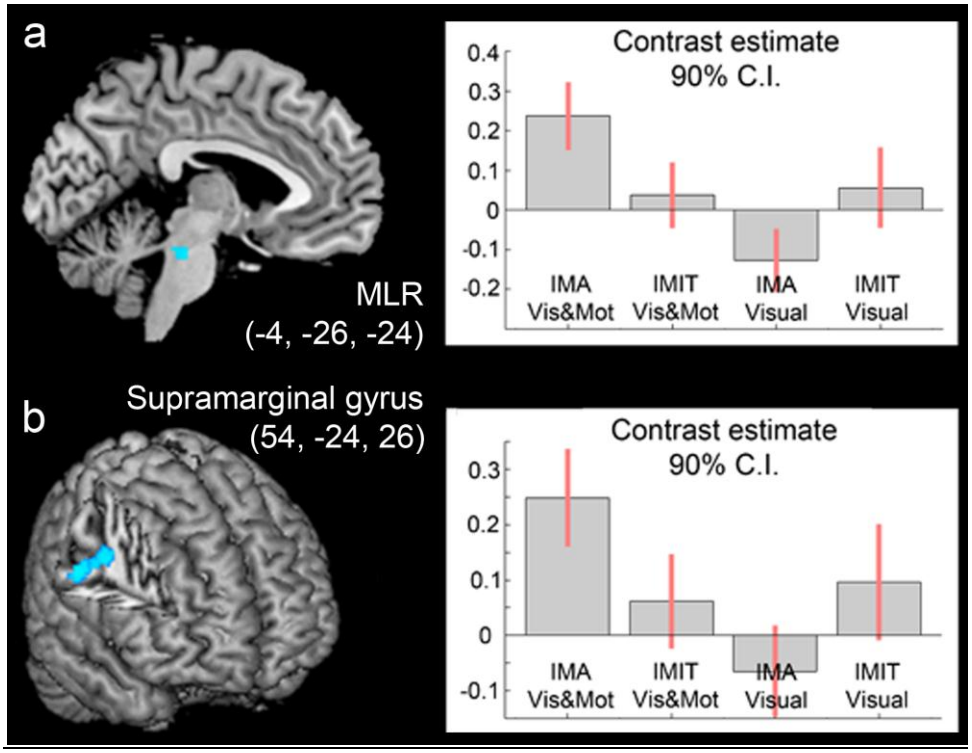


Figure 3.6 Most recruited areas during imagery in comparison to the imitation involving the midbrain (compatible with MLR) and the parietal operculum and supramarginal gyrus, as measeured by the interaction between the imagery (IMA) and imitation (IMT) in the visual&motor (vis&mot) and visual condition, obtained by imagery > imitation condition

Brainstem analysis on minimally smoothed data

The involvement of MLR reported in our whole-brain analysis appears to be trustworthy, since it has been previously reported in fMRI neuroimaging studies on MI for gait related tasks in whole-brain analysis [142, 167] (see Chapter 1 and the introduction of this chapter). In addition the whole-brain normalization procedure of SPM [161] has given good results with regard to brainstem structures [168]. However, in order to exclude the possibility that our activation in the brainstem could be an artifact consequent to the standard smoothing in the pre-preprocessing analysis, we conducted an additional analysis on the brainstem data, applying a minimal amount of smoothing (2x2x2 mm, see [168]). A brainstem mask [169] was used to mask this second-level full factorial ANOVA. The local maximum (x = -4, y = -26, z = -22; Z = 3.30, $p < .001$) obtained from the crucial interaction analysis ('Visual&Motor > Visual' x 'Imagery > Imitation') was actually the same obtained by the previous analysis, smoothed with the 10x10x10 filter (x = -4, y = -26, z = -24). This result confirms the higher involvement of the MLR in the imagery condition with respect to the imitation condition in our vitual walking task.

Preliminary feet movement analysis

The main effect for feet movement (overall main effect, 1 1 1 1 contrast) identified a network of the SMA and paracentral lobule, the Rolandic opercular and superior temporal gyri, the basal ganglia and the cerebellum (see Table 3.5 APPENDIX A and Figure 3.4b). No effects were reported for the direct comparison of the gait imagery against the standing still imagery in the feet movement (Walk (Mov+)> Stand(Mov+) contrast) for both imagery and imitation conditions, when they were considered as main effects or tested separately in the imitation an imagery condition. The main effect imagery/imitation and the interaction effects were also not significant.

3.6 Discussion

The purpose of the study was to identify the degree of neurofunctional overlap between motor imagery as compared with action observation in a gait-related task. In the current literature, the concept of common *motor representational space* for MI and AO is accepted. However, the proofs in favour of this idea are mainly based on tasks involving the upper limbs [170] [171], with lack of information on the lower limbs and especially gait.

To test this hypothesis, we created an "imagery" (MI) and an "imitation" (AO) paradigm using two sets of visually matched virtual walking stimuli (moving naturalistic paths) [97, 156-158] and tested their effects on a group of healthy elderly (see paragraph 3.3 of this Chapter). More specifically, our experimental design was created with two main purposes: (i) to test whether MI or AO for gait-related tasks could evoke activation in the cortico-subcortical gait network as described by a previous study [43, 172-175]; (ii) to observe the possible modulation of AO on the neural network related to the MI task alone. In addition, during the presentation of half of the stimuli, moving and stationary, the subjects were required to execute an ankle dorsiflexion for both the imagery and imitation tasks: the purpose was to isolate the motor network involved in the real movement and define the degree of overlap with those specific for MI and AO. A consistent correspondence has been observed between the neurofunctional network engaged in the dorsiflexion when compared to the activations involved in the imagery condition: a wide cortico-subcortical network including SMA, paracentral lobule, basal ganglia, thalamus and the cerebellum. However the activation reported for the imagery condition were not limited only to those involved during the ankle dorsiflexion, comprehending also the premotor cortex and posterior parietal cortex. The activations reported for imagery were substantially different from the imitation condition: the imagery task recruited a more extensive cortico-subcortical network comprising two crucial clusters not involved in gait mental imitation in the right supramarginal gyrus and in the brainstem (clusters compatible

with the mesencephalic locomotor region (MLR) and pontine reticular formation as described by Jahn [142], see Chapter 1 and Paragraph 3.1).

Neural correlates of the "virtual walking" task

The possibility to understand the neural network involved while we walk in open space appears to be difficult in an fMRI context, due to the strong physical limitations of the instrumentation and the absence of the sensorial and physical feedbacks that play an important role in the constant monitoring of locomotion. Despite these problems, the results obtained using the "virtual walking stimuli" could be enlightening: the comparison of the walking condition against the standing-still condition allowed to single out the combined explicit MI with the observation of a moving scenario, which was filmed on purpose from a first-person point of view to give the participant the perception of watching with their own eyes. Independently from the imitation or imagery conditions, a widespread fronto-temporo-parietal network was reported, including the premotor and motor cortices involved in the motor control of the inferior limbs. Although the reported temporo-occipital-parietal activations are consistent with the elaboration of a complex visual stimulus (such as the virtual walking task), the interpretation of these activations could not be limited to visual elaboration: temporo-parietal and occipital brain regions, and their connections to the frontal lobe, might be involved in the modulation of gait patterns based on visual environmental cues, aimed at achieving a dynamic and efficient integration of body movements in the outside world.

Results reported in literature for lesion data are in accordance with our interpretation the: damages to the parietal white-matter and in the splenium of the corpus callosum were positively correlated with impaired mobility [143, 144]. In addition, the age of our participants could justify the remarkable contribution of posterior multisensory areas: as previously explained (see Chapter 1 and the introduction paragraph of this chapter), locomotion control could be really demanding in the elderly, as observed for other complex motor behaviour, and it could require the compensative intervention of a more extended network, as proposed by the scaffolding model of the aging brain [130, 148-151]. Since it appears that the elderly heavily rely on sensory information from the environment to organize locomotion [148], it is possible that the richness of the environmental cues of our "virtual walking" paradigm could be particularly suitable for evoking MI in our participants.

Our paradigm design allowed us to isolate the motor component of virtual walking, since we were able to cancel the effect of both the visual stimulation and the motor activation due to the simple dorsiflexion.

From the visual&motor > visual comparison, a clearly motor network was identified that, apart from being consistent with the previous literature, was also somatotopically accurate [97, 141, 142]

[43]. Specific motor and sensory cortices mapping the lower limbs, lateral premotor areas, and SMA were reported. The associated cerebellum and the brainstem activation confirmed that our virtual walking motor imagery task engaged both cortical and subcortical networks implicated in locomotor control [42, 43, 172]. The SMA involvement appears consistent with the literature postulating that this area is responsible for gait initiation and the modulation between the subthalamic locomotor region and the MLR: this interpretation is in accordance with the symptoms involved in "gait apraxia", a typical consequence of SMA lesions (see Chapter 1 and paragraph 3.1).

The addition of ankle dorsiflexion allowed us to exclude, from the virtual walking network, the areas specifically involved in this explicit movement, primarily represented by the SMA and the motor cortices of the medial wall and the cerebellum (see Figure 3.4). A large network including the lateral pre-motor (LPM) and lateral dorsal parietal cortices (LDP) appears to be specifically involved in the organization of motor plans, integrating the internally generated program with the environmental cues. These results are consistent with the clinical literature reporting how LPM (involved in gait imagery but not in the ankle dorsiflexion) lesions reduce the ability to adapt gait to turns or narrow passages [17] and cause poor responses to external cues (see Liston [18] for a review). In addition, LPM activation is responsible for ameliorations in gait initiation in Parkinson's disease patients if they are visually guided [173, 174] .

In summary, based on our data, the MI process triggered by the virtual walking task could be interpreted as an imagined counterpart for the motor control engaged during an adaptive gait behaviour more than a simple and schematic feet movement: the activations evoked by our imagery task were consistent but not limited to the network of activations involved in the real execution of the ankle dorsiflexion.

Action observation and "internally generated" imagined gait patterns.

There is a consistent amount of literature reporting somatotopically organized motor responses for action observation tasks in heterogeneous samples [88, 176, 177]. These effects have been associated to the so-called "mirror motor network": a fronto-parietal system able to absolve the double role of responding to both executed and observed actions [83, 85-87, 178]. However, the knowledge on the mirror motor system has been almost completely based on evidence reported for actions executed with the upper limbs [88]: the action of grasping is one of the typical selected motor performances [179, 180]. In the few studies exploring the effect of AO for gait-related tasks, the engagement of motor activation was only reported when the observation was combined with an explicit motor imagery task [181, 182]: starting from here, we wondered whether a task requiring

the participant to imagine imitating an actor's action, presented while walking, would have been able to modulate an explicit MI task. Based on the results obtained, since a specific network for the imitation task has not been recognized, we might suppose that if a facilitatory effect of AO exists this could be represented by the reduced activation of the network involved during the imagery task: several of the gait-related networks were less activated during the imitation task. Above all, the imagery condition recruited the same cortico-subcortical networks involved in locomotion, while the activations these activations were not present in the imitation task. In addition, direct comparison between the motor components of Imagery and Imitation (assessed as an interaction effect) showed how MI was associated with a stronger recruitment of the supramarginal gyrus and brainstem.

The role of the supramarginal gyrus has been considered as the neural correlates of virtual sensory information forwarded by SMA related to willed generation of virtual motor commands action as a consequence of the virtual and was reported for both upper and lower limbs movements [64, 74, 175, 183]. Thus, the supramarginal gyrus increased activation has been associated with the use of motor strategies to solve motor imagery tasks [184]. The supramarginal gyrus might be involved in the integration and manipulation of sensori-motor information during the mental rehearsal of an action, a process that might not be involved during imitation.

The role of the brainstem and the MLR, as extensively explained in the previous chapter, is especially related to the function as a pacemaker to regulate the rhythm of locomotion and coordinate gait initiation, integrating the higher level motor programs with the automatic loops defined by the spinal central pattern generator (see Chapter 1).

Altogether, our data indicate how the imagery task effectively emulates the articulated neural schemes for the proper rehearsal of motor programs needed for walking, from the premotor cortices down to the supraspinal centers that provide internally generated gait patterns.

In comparison, the activation reported for the imitation task, even if involving part of gait-related networks such as the paracentral lobule, SMA and cerebellum [98, 152, 153], proved to be more predominantly visual. The most relevant difference between the imagery and imitation conditions is the absence of activations involving the right parietal and brainstem regions. This leads to different possible interpretations of these results. The first interpretation could be that, since the brainstem, and especially the MLR, is related to the concept of internal pacemaker, the focus could have moved from an internally generated pattern to an externally generated one, bases on the dynamic of the presented actor. In a second interpretation, the absence of brainstem activation could have a different meaning: imitation could be a process that can bypass the subcortical structure, substituting the internal rhythm generator with those of the model observed. This interpretation is in

accordance with rehabilitation studies that have observed amelioration through AO tasks in patients with freezing of gait [185, 186], who are known for poor functioning of the brainstem locomotor regions (see Grabli [187] for a review).

CHAPTER 4 – *The functional brain correlates of motor imagery and imitation of gait in gonarthrosis: an fMRI comparative study with age matched normal controls.*

In this chapter I will present the second fMRI study of my thesis: the data obtained from the experiment on the healthy elderly group described in the previous chapter (see chapter 3) have been compared to the data of a group of patients candidates for a TKA (see chapter 2 for a description of the pathology) that participated to the same protocol. The purpose of this analysis was to use the osteoarthritis as a model of a functional limitation of peripheral origin, to test whether it might modify the neurofunctional networks involved in the fMRI paradigm presented in chapter 3: the imagery and the imitation tasks.

4.1 Introduction

The neural plasticity induced by skills acquisition through physical exercise led to the hypothesis that, based on the same principle, a reverse effect could be observed as a consequence of disuse. Different TMS studies has reported size reduction of the primary motor cortex (M1), as mapped using motor evoked potential (MEP) due to physical limitations: Liepert [188] reported a shrinking effect on M1 after the ankle immobilization, due to injury, in a group of neurologically healthy subjects. Using the same methodology, effects downsized dimensions of the motor and sensory cortex have been reported for temporary anesthesia on the forearm [189], limb amputations [190], and spinal cord injuries [191].

The fMRI has also been used to test the effects of immobilization, allowing to observe the cortical reorganizations on the motor and sensory cortex due to a period of immobilization that could last for weeks [192], days [193], and even hours [194]. These studies focused on the upper limbs and the contralateral hemisphere to the immobilized limb, but bilateral effects have been also reported in successive studies [195].

Similarly to the functional counterpart, cortical changes due to immobilization have been reported at a morphological level as demonstrated by Langer and colleagues [196], who described the longitudinal effects on cortical thickness and fractional anisotropy in patients requiring surgery and the consequent upper-limb immobilization of 14 days.

The behavioral effects due to immobilization or disuse are reflected in motor imagery tasks, as described in Chapter 2. The effects of immobilization on MI have also been tested from the neurofunctional point of view: in a multimodal study, Burianova and colleagues [197] measured the immobilization-induced brain plasticity of a hand describing reduced activation in the controlateral motor and sensory cortex for only imagined movement, as compared to the activity reported for the free hand.

There is a lack of literature on the effect of functional limitation for motor imagery of gait related tasks; in this study we wanted to test the effect of the motor functional reduction due to gonarthrosis for patients candidates to TKA (see chapter 2) on the gait related networks individuated by the previous study in chapter 3. Also as for the healthy subjects of the previous chapter we compared the effect of pure motor imagery and its combination with action observation.

4.1 Aims of the study

The purpose of this study was to compare the population of healthy subjects presented in chapter 3 with a group patients candidate for TKA (see chapter 2 for a description of the pathology).

The patients participate to the same paradigm described in chapter 3: our intent was to evaluate the possible neurofunctional modulation caused by the peripheral functional limitations of our patients on the networks involved in the two conditions proposed, imagery and imitation.

4.2 Materials and methods

Sample

22 healthy subjects (women= 9; age: mean = 66, 64, SD= 7,05; educational level: mean= 13,27, DS= 4,24) and 22 patients (women= 16; age: mean = 68, 18, SD= 7,05; educational level: mean= 8,45, DS= 3,4) participated to the fMRI protocol. The patients have been recruited the day before surgery at the Istituto ortoperdio Galeazzi IRCCS. Out of 37 patients tested, data from 22 patients only could be analyzed while the other have been discarded due to motion artifact (see methods). All participants were right-handed as confirmed by the Edinburgh Handedness Inventory [126], and reported normal or corrected-to-normal vision. The 22 patients recruited were not the same described in chapter 2, so their pathology was referred also to the right leg.

The experimental protocol was approved by the Local Ethics Committee (Comitato Etico Azienda Sanitaria Locale Città di Milano), and was carried out in accordance with the ethical standards of the 1964 Declaration of Helsinki.

In order to participate both the groups had to comply with the same criteria of the previous study (chapter 3). All participants were without medical history of neurological or psychiatric disorders and provided their informed written consent to take part in the fMRI study. To exclude age-related cognitive deficits, all participants also completed the Mini-Mental State Examination, a test providing a summary index of cognitive functioning: it proved that no participant showed signs of cognitive impairment (all scores > 24). These results were consistent with the performances at the Raven's Coloured Progressive Matrices test [128], used as integrated measure to assess the cognitive functioning of the participants.

Finally, participants also completed the Vividness of Movement Imagination Questionnaire (VIMQ, [129]), a self-report questionnaire on explicit motor imagery abilities (the mean scores imagery indicative of good self-reported motor imagery abilities, are reported in table 4.1). While the two group were age matched (**U= 212.500; Z= -0.694; p= .488**) the educational level resulted significantly different (**U= 163,000;Z= -3,556; p= .000**). For this reason the educational level has been considered a regressor of no interest in the analysis (see the analysis paragraph).

All the data are reported in table 4.1

Table 4.1 descriptive data of the two groups . Patients (PT), healthy subjects (HS)

		age	education	MMSE	Raven	VMIQ 1st	VMIQ 3rd
PT	mean	68,18	8,45	28,91	31,83	52,15	54,35
	sd	8,48	3,40	1,23	3,84	13,86	21,02
HS	mean	66,64	13,27	28,92	32,70	48,00	48,56
	sd	7,05	4,24	1,55	3,98	19,50	20,80

fMRI protocols and data acquisition and preprocessing.

The fMRI protocols was the same described in the previous chapter so I will not re describe the paradigm (see the materials and method paragraph of chapter 3).

As for the data acquisition and the preprocessing at first level, we used the same procedure applied on the data in chapter 3 with only one exception: the settings of ART (ART, Whitfield- Gabrieli, http://www.nitrc.org/projects/artifact_detect) for the control of the movement artifacts described for healthy subjects study were too restrictive for the patients: due to the gonartrhosis the patients moved more than the healthy subjects during the ankle dorsi-flexion causing an higher level of

artifacts. In order to have comparable data, the level of control by ART was reduced, leaving a 1-mm scan-by-scan comparison but only considering the global signal level, with an extended threshold of 9 standard deviations. The data of both the patients and the healthy subjects have been re-analyzed with the new parameters.

Statistical analyses of the fMRI data.

Similarly to chapter 3 a two-step statistical analysis, based on the general linear model (GLM), was performed, following the same steps: the analysis of the BOLD signal was convolved with a canonical haemodynamic response function [164] and followed by the application of an High-pass filtering (128 s) to remove artifactual contributions to the fMRI signal.

The realignment parameters calculated in the preprocessing step were added in the GLM as regressors of no interest.

The condition-specific effects calculated for the fixed-effect analysis were the same used for the healthy subjects analysis (see chapter 3): we isolated the specific effect of imagery of standing (Stand) and imagery of walking (Walk). Each effect was considered twice based on the association of the dorsi-flexion (walk (Mov+); stand (Mov+)) or its absence ((walk (Mov-); stand (Mov-)). Two classes of events were also defined as specific regressors of no interest: (i) the presentation of the 3 s written instruction image ("resting feet" or "move your feet"), and (ii) the presentation of the alerting questions about the videos and the time-window to record participants' response (8 s in total).

The individual contrast images generated in the first-level analysis were entered into two separate second-level full-factorial ANOVAs that conformed to random effect analyses [165, 198], to allow for generalisation to the population level using group-based statistical inferences.

The Feet movement analysis was not replicated in this study while the virtual walking analysis has been divided in two separated analysis for the imagery and the imitation conditions.

Virtual walking analysis for the imagery condition

In this analysis we investigated the neural correlates of motor imagery of gait during the Virtual Walking by comparing the brain responses of the two groups for the "imagery" condition. For both the patients and the healthy elderly groups the contrasts introduced in the second level analysis were obtained subtracting from the effects of the "Walk" condition the corresponding "Stand" baseline: this was replicated for both the variants of the stimulus, with and without the association of the dorsi-flexion.

The results were the following contrasts:

- Walk(Mov-) > Stand(Mov-) in the imagery run of the patients group
- Walk(Mov+) > Stand(Mov+) in the Imagery run of the patients group
- Walk(Mov-) > Stand(Mov-) in the imagery run of the healthy subjects group
- Walk(Mov+) > Stand(Mov+) in the imagery run of the healthy subjects group

As described in chapter 3 the contrast 'Walk(Mov-)' > 'Stand(Mov-)' represent the 'visual&motor' effects associated with both visual and (implicit) motor processes involved in the virtual walking while the contrasts 'Walk(Mov+) > Stand(Mov+)' represent the 'visual' effect cause the motor component, associated with explicit feet movements, were cancelled-out by the baselines.
The four con-images from each participant derived from the aforementioned first-level comparisons were entered into a second-level full-factorial 2x2 ANOVA having two factors: Components (visual&motor vs. visual) as within-subject factor and group (healthy subjects vs. patients) as between-subjects factor. The educational level was inserted and regressor of no interest in the analysis.
The design matrix was thus organized as follows:
Patients (visual&motor); patients (visual); healthy subjects (visual&motor); patients (visual);

We calculated the following linear contrasts to generate SPM[t] maps:

1) Main effect of group, healthy subjects > patients: contrast -1 -1 1 1
2) Main effect of group, patients > healthy subjects: contrast 1 1 -1 -1
3) Effect of group in the Visual&motor condition, patients > healthy subjects: contrast 1 0 -1 0
4) Effect of group in the Visual&motor condition, healthy subjects > patients: contrast group -1 0 1 0
5) Effect of group in the Visual condition, patients > healthy subjects: contrast 0 1 0 -1
6) Effect of group in the Visual condition, healthy subjects > patients : contrast group 0 1 0 -1
7) Interaction effects between components (visual&motor vs. visual) and group (healthy subjects vs. patients): contrasts (1 -1 -1 1) and (-1 1 1 -1)

Contrasts at point 2, 3 4 and 5, for both imagery and imitation, were masked by the respective simple effects of each condition (thresholded at $p < .001$) to only include voxels significantly activate during the conditions of interest. All analyses are conducted at the whole-brain level,

thresholded at $p < .001_{uncorr}$, and only peaks in clusters significant at $p < .05$ for their spatial extent are reported. The regional effects that also survived a voxel-wise and cluster-wise FWE correction are reported in the Tables. APPENDIX B

Virtual walking analysis for the imitation condition

The analysis for the imitation condition was constructed similarly to the imitation, obtaining the following contrast:

- Walk(Mov-) > Stand(Mov-) in the imitation run of the patients group
- Walk(Mov+) > Stand(Mov+) in the Imitation run of the patients group
- Walk(Mov-) > Stand(Mov-) in the imitation run of the healthy subjects group
- Walk(Mov+) > Stand(Mov+) in the imitation run of the healthy subjects group

Similarly to the imagery condition, we calculated linear contrasts to generate SPM[t] maps as follows:

1. Main effect of group, healthy subjects > patients: contrast -1 -1 1 1
2. Main effect of group, patients > healthy subjects: contrast 1 1 -1 -1
3. Effect of group in the Visual&motor condition, patients > healthy subjects: contrast 1 0 -1 0
4. Effect of group in the Visual&motor condition, healthy subjects > patients: contrast group -1 0 1 0
5. Effect of group in the Visual condition, patients > healthy subjects: contrast 0 1 0 -1
6. Effect of group in the Visual condition, healthy subjects > patients : contrast group 0 1 0 -1
7. Interaction effects between components (visual&motor vs. visual) and group (healthy subjects vs. patients): contrasts (1 -1 -1 1) and (-1 1 1 -1)

Contrasts at point 2, 3 4 and 5, for both imagery and imitation, were masked by the respective simple effects of each condition (thresholded at $p < .001$) to only include voxels significantly activate during the conditions of interest. All analyses are conducted at the whole-brain level, thresholded at $p < .001_{uncorr}$, and only peaks in clusters significant at $p < .05$ for their spatial extent are reported. The regional effects that also survived a voxel-wise and cluster-wise FWE correction are reported in the Tables. APPENDIX B

4.3 Results

Behaviour

Differently from the results obtained in chapter 2, the performances in both the TUG and the simulated walk tasks of this sub-sample of patients resulted significantly different respect their healthy controls, considering the same threshold of $p \leq .004$. These significant results were, obtained for both the executed (TUG (**U=30,000;Z= -4,976; p=.000**); simulated walk(**U=115,000; Z= -2,891; p= .003**)) and the imagined (TUG (**U=87,000;Z=-3,638;p=.000**); simulated walk(**U=79,000; Z= -3,828;p= .000**)) conditions (figure 4.1,4.2). However when comparing the subtractions "imagination-execution" (see chapter 2) to test the level of isochrony the trend obtained did not differ from the previous sample of patients (TUG (**U= 131,500; Z= -2594; p= .009**) ;simulated walk(**U= 136,000;Z= -2488; p= .013**) for a threshold of $p \leq .008$)(figure 4.3).

The behavioural profile of this sub-sample of 22 patients was virtually identical to the one described in chapter 2. To summarize, while showing signs of a motorically driven motor imagery ability, the patients' imagination was comparatively faster, once the speed of the motor execution was taken into account, to suggest that their imagination had not taken their motoric limitation fully on-board.

Yet their performance in the more demanding TUG and "simulated walk" tasks display a high correlation between the imagined and the executed versions of the tasks (respectively **ρ= ,768**, p=.000** for the TUG test and **ρ= ,877** p=.004** for the simulated walk**).**

Figure 4.1 performances, expressed in M/S, in the TUG task, imagined and executed for the healthy subjects (blue) and the patients (red). The differences between the two groups resulted statistically significant for both the conditions. For each task the standard error mean is also reported.

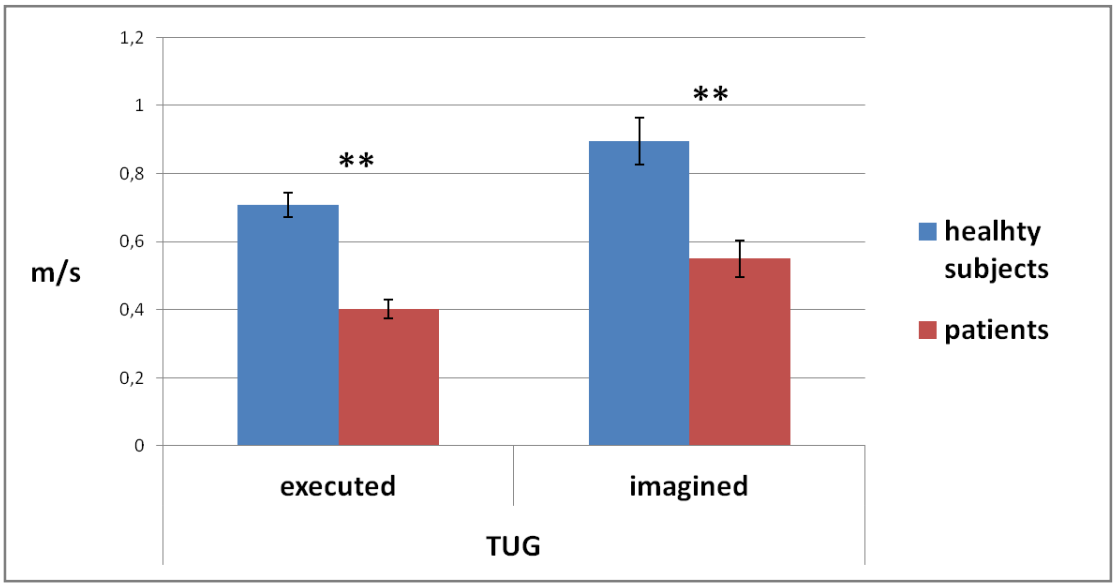


Figure 4.2. Simulated walk task. Mean of the performances, expressed in seconds, for the executed and imagined conditions for the patients (red) and the healthy subjects (blue). For each task the standard error mean is also reported. The differences between the two groups resulted statistically significant for both the conditions. For each task the standard error mean is also reported.

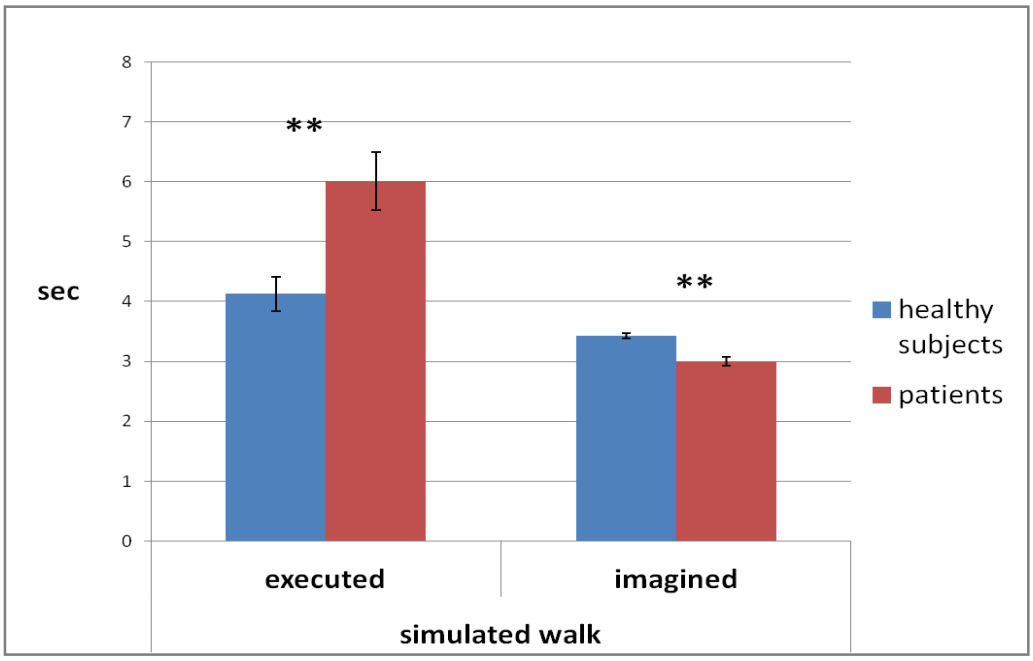
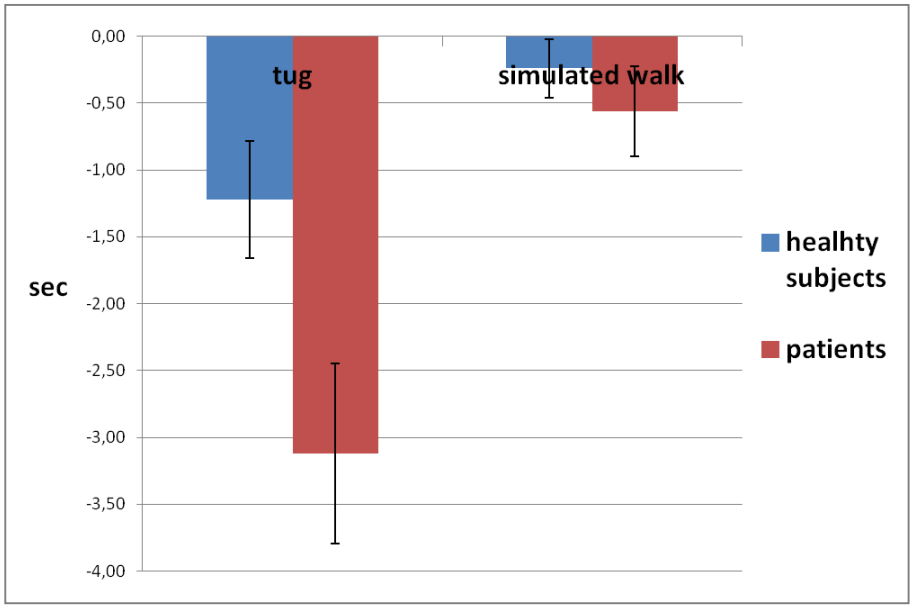


Figure 4.3. level of isochrony: mean of the differences for each task, expressed as RTs in seconds rather than speed, for the healthy subjects (blue) and the patients (red). The performances did not result significantly different for both the tasks. For each task the standard error mean is also reported.



fMRI results:

Imagery condition

Main effects of between group comparisons

Patients > healthy subjects (1 1 -1 -1) and healthy subjects > patients (-1 -1 1 1):

The analysis for the group main effect in the imagery condition identified the activation in the putamen for the healthy subject main effect while for the opposite condition (1 1 -1 -1) no results survived to the correction. The results are reported in table 4.2. APPENDIX B

Visual&motor component effect

healthy subjects > patients (-1 0 1 0) masked over the simple effect for the healthy subjects (0 0 1 0)

The differences for the visual&motor component effect for the healthy subjects are reported in table 4.3. APPENDIX B (see also figure 4.4., 4.5.). The activations reported involve the SMA an Cingulum bilaterally, a large parietal cluster involving the superior and inferior parietal gyrus, mainly left, in addition activations were reported in the right precuneus and left cuneus. Involved in the networks result also part of the right middle temporal gyrus and a large occipital cluster comprehending the superior occipital gyrus, bilaterally, the right middle occipital gyrus. Consistent activation were also reported in subcortical circuit involving an extent part of the putamen and reaching also the brainstem and cerebellum.

Patients > healthy subjects (1 0 -1 0) masked over the simple effect for the patients (1 0 0 0)

No voxels survived for the opposite contrast (patients >healthy subjects).

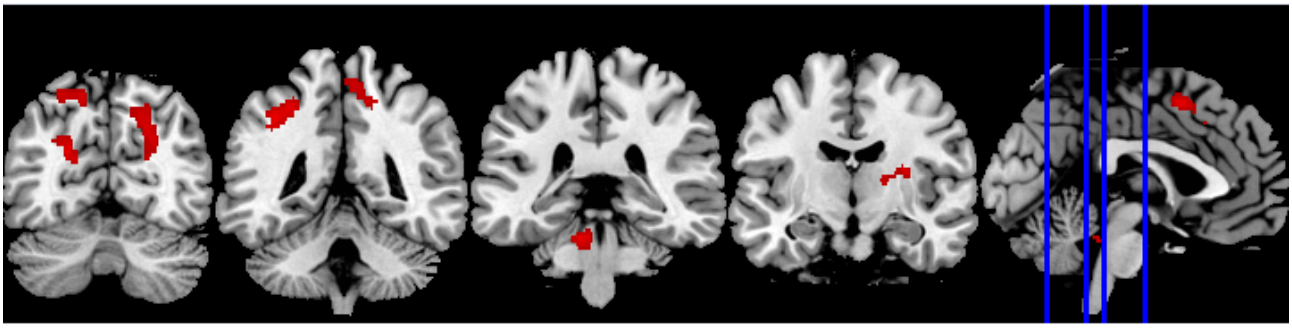


Figure 4.4 the posterior networks of activations identified for the visual&motor component in the healthy subjects groups as compared to the patients group (healthy subjects > patients (-1 0 1 0) masked over the simple effect for the healthy subjects (0 0 10). Coronal view.

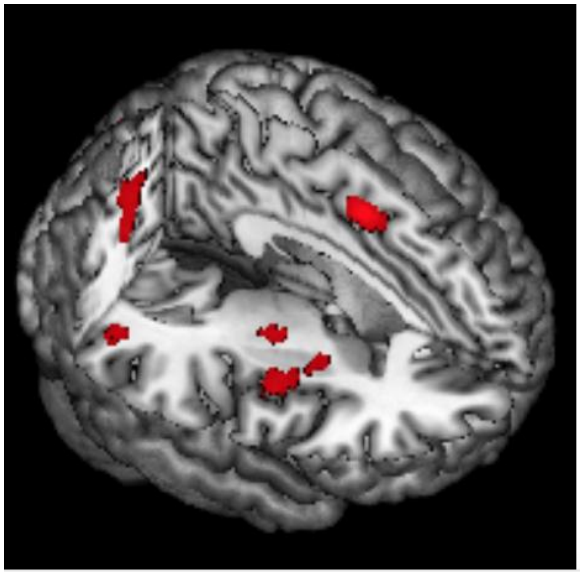


Figure 4.5 activations for the effect of visual&motor for the healthy groups (healthy subjects > patients (-1 0 1 0) masked over the simple effect for the healthy subjects (0 0 1 0). The image underline the frontal and subcortical activation.

Visual component

patients > healthy subjects (0 1 0 -1) masked over the simple effect for the patients (0 1 0 0)

healthy subjects > patients (0 -1 0 1) masked over the simple effect for the healthy subjects (0 0 0 1)

In both the direction of the comparison no effects emerged for the visual components of the two groups

Interaction effects

'Visual > visual&motor' x 'patients > healthy subjects' (-1 1 1 -1)

This interaction effect, that compared the visual[1] over the visual&motor components for the patient respect the healthy subjects, identified an extended cortical posterior network involving the right post central gyrus, a large cluster in the left parietal lobe, part of the left superior temporal and lingual gyrus and finally the left middle occipital gyrus. Subcortical activation were reported in the thalamus, the hippocampus, the vermis and the left cerebellum and the brainstem bilaterally. In addition some activation were reported in the white matter (table 4.4 APPENDIX B, figure 4.6). In other words, these regions are more active in the patients when they are also moving their feet (in spite of the subtracted baseline) while in the normal controls the opposite applies.

'Visual&motor > visual' x 'patients > healthy subjects'(1 -1 -1 1)

No voxels survived the opposite interaction.

[1] In the condition called "visual" subjects were moving their feet both in the experimental condition and in the baseline.

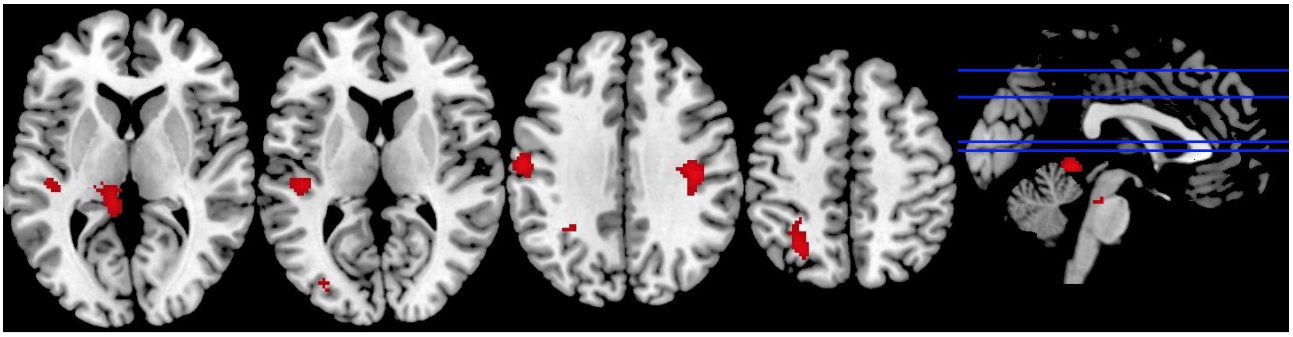


Figure 4.6 interaction effect for the imagery tasks 'visual >visual&motor'x 'patients > healthy subjects'(-1 1 1-1). Involvement ot the brainstem and the cerebellum (up), assail view. Activations of the posterior networks and the subcotical components (down) .

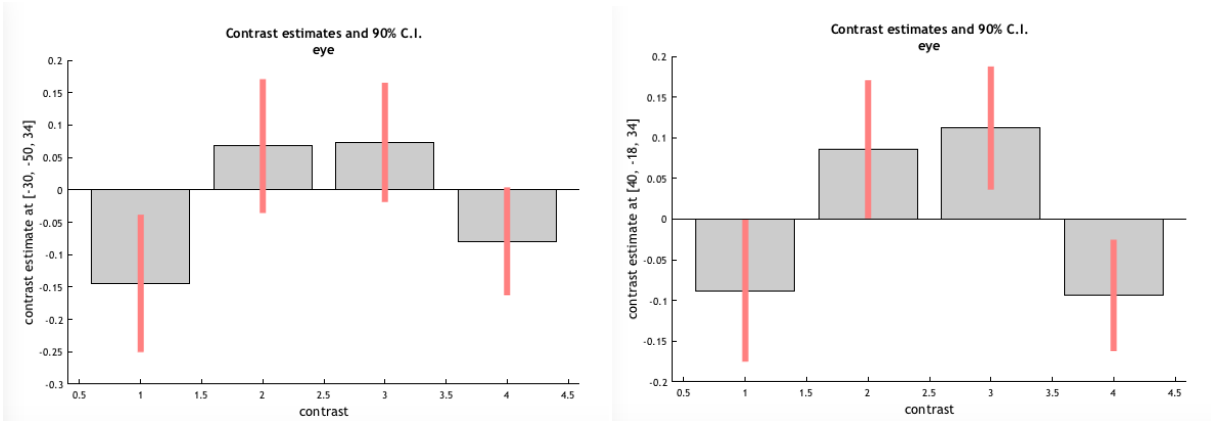


Figure 4.5 The histogram represent the two component of the imagery task, the columns are organized as follows:

1. **First column: visual&motor component for the patients**
2. **Second column: visual component for the patients**
3. **Third column: visual&motor component for the healthy subjects**
4. **Fourth column: visual component for the healthy subjects**

The two trend reported are the similar activation for the visual component in the patients group as compared to visual&motor component of the healthy subjects as plotted on the effect during all the task (eye contrast): on the left is the trend for the parietal cluster, on the right the trend for the post central cluster.

Imitation condition

Group main effects

patients > healthy subjects (1 1 -1 -1) and healthy subjects > patients (-1 -1 1 1):

In the imitation condition no voxels survived for the group main effect, in both the direction.

Visual&motor component effect

patients > healthy subjects (1 0 -1 0) masked over the simple effect for the patients (1 0 0 0)

healthy subjects > patients (-1 0 1 0) masked over the simple effect for the healthy subjects (0 0 10)

The visual&motor component for the healthy group (healthy subjects > patients) identified two principal clusters: the first was a small cluster that involved the paracentral lobule bilaterally, the second instead was a large cluster that comprehended the left superior and middle parietal gyrus (table 4.5 APPENDIX B, figure 4.7)

For the opposite contrast (patients > healthy) no voxels survived the analysis.

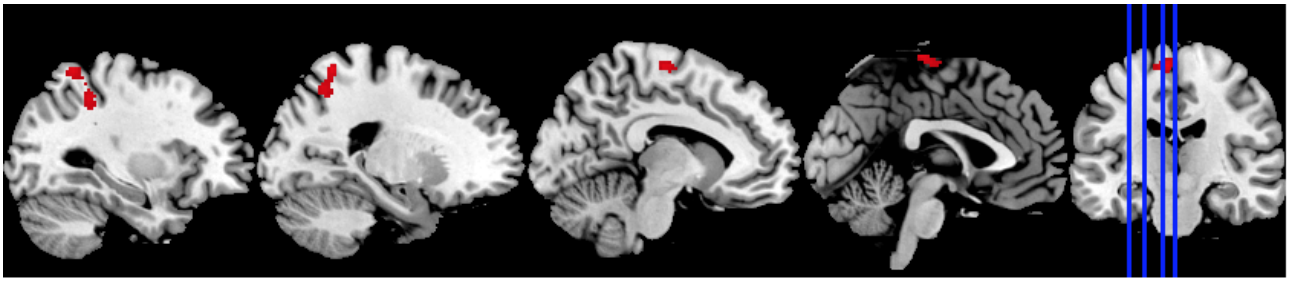


Figure 4.7 activation for the visual&motor component in the imitation condition for the healthy group (healthy subjects > patients)

Visual component

patients > healthy subjects (0 1 0 -1) masked over the simple effect for the patients (0 1 0 0)

healthy subjects > patients (0 -1 0 1) masked over the simple effect for the healthy subjects (0 0 0 1)

In both the direction of the comparison no effects emerged for the visual components of the two groups

Interaction effect

'visual&motor > visual' x 'patients > healthy subjects' (1 -1 -1 1)
'visual > visual&motor' x 'patients > healthy subjects' (-1 1 1 -1)

Only the second interactions analysis for the visual component effect identified some activation: differently from the previous analysis this interaction reported activation mainly in the white matter, with the only exception of two cluster involving the lingual gyrus and the cingulum. The white matter activation comprehended the the retrosplenial part of the corpus callosum, the optical radiation, the arcuate fasciculus and the occipito frontal fasciculus, all on the left hemisphere. (table 4.6 APPENDIX B)

4.4 Discussion

The purpose of this study was to evaluate whether physical limitation caused by a pure peripheral disease, such as osteoarthritis, would be able to modulate the neurofunctional responses of a group of candidate patients for TKA for motor imagery gait-related tasks. In the previous chapter (see

Chapter 3), two gait-related tasks were constructed using a set of virtual walking stimuli: we created a pure motor imagery (imagery condition) and a second condition that combined the use of motor imagery and action observation (imitation condition). In addition, for half of the stimuli the patients were asked to execute an ankle dorsiflexion while performing both the imagery and the imitation tasks. In both the imagery and imitation condition, each stimulus was anticipated by a matched baseline. The effect of lower limb movements was cancelled out, because it was present both in the experimental conditions (Walk) and in their respective baselines (Stand), and so it was subtracted by creating the contrast images: for this reason, the contrast 'Walk(Mov+) > Stand(Mov+)' was defined as 'visual', because it identifies the activations related to the visual processes of in-motion stimuli applied in the Virtual Walking tasks, without the motoric component. On the other hand, the contrast 'Walk(Mov-)' > 'Stand(Mov-)' was defined as a visual&motor effect, because it represents the combined visual and (implicit) motor processes involved in the virtual walking imagery, since the possible noise due to the visual information was cancelled by the use of the baseline, like the movement execution noise.

As discussed in Chapter 3, our "imagery" (visual&motor>visual) condition was able to activate a widespread gait-related network consistent with the real gait in healthy subjects, involving a cortical network of motor areas and areas involved in sensory motor integration. Furthermore the task was able to evoke response in the subcortical structure, also involving the brainstem region compatible with the mesencephalic locomotor region, and the reported activations were not limited to those caused by the ankle dorsiflexion, allowing us to postulate that the task was able to emulate locomotion in an open space.

On the contrary, the "imitation" (visual&motor>visual) tasks appears to be less consistent with gait execution: the major difference to the "imagery" was the absence of parietal and brainstem activations. The results have been interpreted based on the functional role of mesenchephalic locomotor region (MLR), that codes the internal pace and so the rhythm of the actual locomotion of an individual: in the imitation task, the request was to imagine imitating the movements of someone else, so the pace in this case was not internal but "imposed" by an external model. We postulated that the "imitation" task was able to bypass the MLR recruitment for this reason. These results were also in line with the activation reported in the cerebellum and the positive effect of observation-based rehabilitation for patients with freezing of gait, a symptom related to the malfunctioning of the MLR[187].

In this chapter both the visual&motor and the visual components of the two groups were compared, separately analyzing the imagery and the imitation condition.

The effect of gonarthosis over imagery

The first analysis conducted was to test group differences in the imagery condition. First we examined the main effect. The first important result was the absence of activation obtained when we searched for activation that distinguished our patients' group from the healthy subjects (patients>healthy subjects): it appears that osteoarthritis did not induce the generation of any compensatory strategy to perform the tasks in our patients' group. On the other hand, the opposite comparison (healthy>patients) found a significant cluster situated in the putamen. This region has been previously reported in tasks comparing the imagination of walking with a condition of rest [43], and has also been reported as a region involved in the activation networks comparing a closed-eye task involving the imagery of running in first person, in comparison with the same condition in third person [199]. An interesting data is that putamen activations relate to gait temporal symmetry: in a group of stroke patients, the lesion to the putamen has been reported to cause asymmetry in the temporal dynamics of gait, expressed as the tendency to rely more on double leg support, reducing the time of unloading over the impaired leg [200]. This appears quite consistent with the gait dynamic of our patients and their functional limitation, who rely more on the leg that should not undergo surgery.

We then examined the effect of group in the visual&motor condition. Again, no sign of compensation was reported for the patients' group and the results identified only a more widespread network of activation in the healthy subjects. These activations included areas directly involved in motor control, such as the SMA, and a wide parieto-temporo-occipital network. These activations are consistent with the network reported for the motor component of virtual walking in Chapter 3. In particular, the parietal cortex plays an important role in sensorimotor integration, especially in the updating of information to maintain an internal representation [201]. In addition, this area is also involved in motor control, as shown by lesion studies [143]. Another relevant reported activation area was the precuneus, previously reported to have a role in the spatial elaboration of motor imagery tasks[202]. Furthermore this contrast also identified a subcortical network involving a large part of the putamen, whose role has been described for the main effect analysis, the brainstem, in a region compatible with the MLR, and the cerebellum.

Finally, no difference between patients and healthy controls has been reported in the visual condition.

On the contrary, the interaction effect reported significant activations at both cortical and subcortical level. An interesting result was that the visual component for the patients' group seems to activate areas that the healthy subjects activate during the visual&motor condition. This was reported more consistently for the postcentral gyrus and the parietal cortex: it seems that the action

execution, even if cancelled out, is able to trigger the activation for our patients in areas that the healthy subjects use during pure imagination.

In summary, the analysis of the imagination condition showed that the functional limitations affecting our patients' group had a negative effect on their imagery performance: no compensatory networks emerged when directly comparing the two group. However, the association with ankle dorsiflexion had a positive modulatory effect in gait specific regions.

The results obtained suggest that the imagery task might be an efficient tool to emulate real locomotion even in patients with marginally deviant motor imagery: however, it might well be that a concurrent minimal motor activity like ankle dorsiflexion might boost the recruitment of crucial cortico-subcortical gait structures, particularly in patients.

The effect of gonarthrosis over imitation

The network activated during the imitation task was smaller than during the imagery task, consistently with the results reported in Chapter 3. Significant differences between the two tasks only emerged in the visual&motor condition. Similarly to the results in the imagery task, these differences were characterized by hypoactivations in the patients' group. No hyperactivations in patients as compared to healthy controls emerged, suggesting the absence of any compensatory process.

The significantly more active network in healthy controls included the paracentral lobule and the parietal cortex, indicating that in the imitation task as well, the network that emerged from the difference between the two groups involved areas connected with movement control and sensorimotor integration. This suggests that the controls solved the mental imitation task by applying a more motor strategy. Moreover, the absence of subcortical activation was consistent with the previous results (see Chapter 3) that demonstrate how the gait-related network was not represented during the imitation condition.

The interaction effect emerging from the imitation task was the same as reported for the imagery condition (visual>visual&motor x patients >healthy subjects).

In conclusion, this study demonstrates that patients with gonarthrosis had (1) behavioral signs of a qualitatively different form of motor imagery, (2) while showing general signs of reduced recruitment of motor specific brain structures during motor imagery per-se (3), and do not show generalized signs of compensation such as those observed, for example, in graceful aging by Zapparoli et al. [130] in the visual cortices. However, (4) the analysis of the modulatory impact of concurrent ankle dorsiflexion over the imaginatory activity showed that the motor imagery networks were available to our patients.

This suggests that, in principle, motor imagery with a minimal concurrent motor task is worth trying out as a complementary rehabilitation strategy in gonarthrosis patients, for example, after surgery.

CHAPTER 5- *Conclusions*

Gait is a highly automatic behaviour. Nevertheless, its organization is only apparently simple and the interpretation of gait as a mere series on spinal reflexes has drastically changed in the last 30 years. In my thesis I described how gait control is divided in different levels, starting from the simple firing of motor neurons at spinal level, to the elaboration of articulate motor programs at cortical level. I explained why the methods of cognitive neuroscience, and especially fMRI, have become relevant for the study of locomotion control, introducing the concept of higher level gait disorders and describing the complex cortico-subcortical networks involved in gait behaviour. The fMRI context poses physical limits to study active locomotion so I've described the methods used in the fMRI studies to overcome this problem, namely motor imagery and action observation. It is widely accepted that both motor imagery and action observation share a common "motor" representational space with actual action execution; up to now however, no studies have directly compared the two tasks to define their similarities and differences for matched stimuli, at least for the behaviour of gait. This was the principal aim of the study presented in Chapter 3: we studied the relation between action execution associated with motor imagery and with action observation for gait-related tasks in a group of healthy elderly. The results obtained demonstrated how motor imagery was effective in the emulation of real gait, involving activation in both cortical and subcortical networks, while imitation recruited activations in a more restricted motor network, involving the cortex and the cerebellum, but not the brainstem. After comparing the imitation and imagery conditions in the healthy subjects, we asked ourselves how patients with gait impairment would perform the same tasks and how their physical limitation would be reflected at a neural level.
Changes in gait abilities accompany humans along their life cycle: gait acquisition requires considerable labour, and then the decay of gait-related skills are one of the defining traits of ageing, particularly in wealthy societies in which the life expectancy is longer than ever imagined. Several causes can affect gait in adult and elderly people: neurological disorders like Parkinson's disease or cerebrovascular diseases are those that probably spring to mind first. Yet, as much as this may have surprised the reader, as discussed, osteoarthritis in the lower limbs is considered the single most important cause of disability and handicap in Western industrialized countries. It is a major cause of musculoskeletal pain, and daily life activities are reduced due to severe functional limitation that

increases for specific actions such as kneeling, stair climbing, walking for long periods of time, or driving, and the movement involved in dressing up or taking care of personal hygiene.

For this reason we decided to study a group of candidates to TKA at neurofunctional level, comparing them with the group of healthy subjects. The neurofunctional study was complemented by a behavioural investigation: we used mental chronometry to test whether our patients would respect the principle of functional equivalence between execution and imagination for gait-related motor imagery tasks. I found that patients with knee osteoarthritis are still capable of motor imagery for the walking behaviour (they show highly significant correlations between the time taken for a real walk and the time taken to imagine the same walk); yet, they seem not to have incorporated their peripheral motor limitation in the walking simulation performed during imagery as they are comparatively faster in motor imagery than the normal controls, once the time taken to walk is subtracted out;

In the fMRI paradigm, the patients' group showed reduced activations at the level of the neural networks recruited for motor imagery and action observation, compared with the healthy group study. These results showed how osteoarthritis had impacted on the inner motor representation. Moreover, no signs of compensatory mechanism attempts were identified, since the opposite statistical comparison (patients > healthy controls) in the same analysis did not present any significant results. Among the different areas that were less active in TKA patients with respect to the healthy controls, the involvement of the putamen was particularly interesting, considering the role played by this area in real locomotion. Putamen lesions in stroke patients cause the alteration of the temporal dynamics of gait, expressed as the tendency to rely more on double leg support. This kind of walking behavior is partially mimed by the TKA patients due to their tendency to rely more on the leg that should not undergo surgery.

However, the analysis of the modulatory impact of concurrent ankle dorsi-flexion over the imagery activity showed that the motor imagery networks were available to our patients. This is an interesting result, since it mimics an effect observed by Frick et al. [203] in developmental cognitive psychology, readapting the classical Piagetian water tilt task. The authors observed how the struggle of 5-year-old children to perform a motor imagery task, due to their still limited abstraction abilities, benefits from an active execution: if the children were allowed to physically execute the task their performances improved during the imagined condition. The authors explain the results assuming that, once the children had performed the task in real life, they could refer to a structured motor scheme while performing the motor imagery task. The similitude with the children's behavior observed in our patients might be explained as a consequence of a degraded motor representation of a once-structured gait behavior: in other words, if children use action

execution to solve a motor imagery task because they have not consolidated their inner motor representations, our patients use the real execution to reactivate previous motor representation that are now less available.

Future directions and scenarios

The experiments described in this thesis have a clear implication as they set the rationale that we are entertaining of following up patients after surgery and test the hypothesis we could observe an evolving scenario along the post surgical recovery. For example we might expect that the initial post-surgery immobilization could be severe enough to allow the update of the still incomplete inner motor representations of our patients, as described in Chapter 2. One possibility is then that the initial post-surgical immobilization could make the degradation of the gait-related motor representation even worse, we could expect the opposite scenario in the long term, after physical rehabilitation: at the end of their post-surgical recovery, the patients might be able to behave like our healthy controls.

The data described in this thesis also laid the theoretical foundation to extend the investigation on the rehabilitation level. The patients may benefit from a rehabilitation based on motor imagery to compensate the temporary disuse of the operated leg. Hopefully, this rehabilitation might be helpful not only for the pure ability of motor imagery, but also for the physical recovery, due to the direct relation with real execution demonstrated by the fMRI data of this thesis. Of course, this rehabilitation will not substitute the physical therapy but it could be used as an enforcer, easily integrated in the recovery plan. In addition, the stimuli used to test our patients at a behavioral and neurofunctional level could be used as a template to create the rehabilitation tools. Subjects could be trained to use the virtual walking stimuli, both in the imagery and imitation conditions, to evoke the physical and kinesthetic sensation related to gait execution. Furthermore a series of eys-closed exercises similar to the imagined TUG test could be useful, with the support of a therapist, to reconstruct the degraded inner representation caused by the reduced motility due to the surgery. Finally the same paradigm described in this chapter could be used to test the effectiveness of this hypothetical rehabilitation protocol.

These are all empirical questions that, together with the research team I belong to, I am currently testing.

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APPENDIX A

Table 3.2 Cortical activation for the overall main effect of virtual walking

Motor component of the virtual walking task.

	Left Hemisphere				Right Hemisphere			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
Inf. frontal orb. gyrus (38)	-36	22	-14	3.5	46	18	-12	3.2$
Inf. frontal op. gyrus	-56	14	2	4.0	56	14	0	5.4*$
Insula (47)	-30	20	-12	3.5				
Sup. frontal gyrus (6)	-22	-12	54	5.2*$	22	-10	56	5.7*$
	-28	0	68	4.2$				
Mid. frontal gyrus (46)	-30	30	16	4.4				
	-34	38	34	4.0				
	-36	40	24	3.6				
	-28	30	24	3.6				
	-30	38	28	3.4				
SMA (6)	-4	-4	66	5.5*$	6	-10	74	5.0*$
	-2	8	56	4.1$				
	-8	2	52	4.0$				
	-14	0	52	3.8$				
Precentral gyrus (6)	-32	-8	52	4.7*$	12	-24	78	4.3$
Paracentral lobule (4)	-4	-24	60	5.2*$	2	-42	68	4.5$
	-8	-30	76	4.3$				
Postcentral gyrus (1/3)	-28	-40	70	4.6$	32	-28	44	4.1$
					32	-36	68	3.2$
Postcentral / Supramarginal gyrus (3/40)					34	-32	42	4.2$
Supramarginal gyrus (40)	-48	-44	28	4.2$	36	-34	38	4.1$
					66	-24	20	3.6$

Motor component of the virtual walking task. (continue)

	Left Hemisphere				Right Hemisphere			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
Sup. parietal gyrus (40)					40	-44	62	4.2$
Rolandic opercular gyrus					54	-26	24	4.4$
Precuneus (5)	-8	-40	66	4.8*$	12	-52	70	4.1$
Mid. temporal gyrus (37/21)	-42	-46	16	3.8$				
	-46	-42	12	3.4$				
	-38	-50	6	4.2$				
Sup. temporal gyrus (42)	-54	-24	14	4.7*				
	-50	-44	18	4.1$				
Sup. temporal pole (38)	-44	20	-16	3.8				
	-54	16	-8	3.5				
Caudate					8	12	-2	3.7
					12	14	-2	3.7
Pallidum					18	2	4	4.4
Thalamus					18	-10	0	4.0
					16	-4	0	3.8
Cerebellum – Vermis					0	-48	-10	5.5*$
Cerebellum – III Lobule					16	-32	-24	6.4*$
Cerebellum – IV-V Lobule	-16	-34	-24	6.2*$	10	-42	-18	4.4$
	-4	-46	-14	5.6*$				
	-24	-40	-28	5.0*$				
	-18	-50	-24	4.6$				
Cerebellum – VI Lobule	-28	-44	-30	5.0*$	28	-48	-30	5.5*$
	-26	-56	-24	4.5$				
	-24	-48	-26	4.4$				

Motor component of the virtual walking task. (continued)

	Left Hemisphere				Right Hemisphere			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
Cerebellum – VI Lobule	-28	-44	-30	5.0*$	28	-48	-30	5.5*$
	-26	-56	-24	4.5$				
	-24	-48	-26	4.4$				
	-8	-60	-22	3.6$				
Cerebellum – IX Lobule	-20	-42	-48	3.8$				
Cerebellum – X Lobule	-20	-34	-40	3.7$				
Cerebellum / Middle cerebellar peduncle	-22	-38	-36	3.7$				
Brainstem					8	-32	-40	3.5$
Corpus Callosum	-14	32	2	4.7*				
Corpus Callosum	-20	34	2	4.8*				

x, y, and z are the stereotactic coordinates of the activations in the MNI space.
Statistical threshold $p < .001_{uncorr}$. Only voxel included in significant cluster are reported.
(*) Z-scores statistically significant also after the FWE (family-wise error) correction.
($) Voxel included in a cluster surviving the FWE-correction at cluster-level.

Table 3.3 Motor components of the virtual walking task (Virtual Walking analysis) (a) in the Imagery condition, i.e. 'Imagery(visual&motor) > Imagery(visual)' contrast; (b) in the Imitation condition, i.e. 'Imitation(visual&motor) > Imitation(visual)' contrast; (c) in common between the Imagery and Imitation conditions, i.e. conjunction analysis 'Imagery(visual&motor) > Imagery(visual)' ∩ 'Imitation(visual&motor) > Imitation(visual)'.

A. Motor component of the virtual walking task in the Imagery condition

	Left Hemisphere				Right Hemisphere			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
Medial orb gyrus					20	30	-10	4.0$
Inf. frontal orb. gyrus (47)					30	26	-14	4.8*$
					38	30	-8	4.0$
					36	30	-4	4.0$
					50	30	-10	3.7$
					44	32	-10	3.7$
Inf. frontal op. gyrus					56	14	0	5.5*$
Insula (47)	-30	20	-12	4.0$				
	-36	2	-14	3.8$				
Insula (38)					44	18	-12	4.0$
Sup. frontal gyrus (6)	-22	-10	52	5.7*$	22	-10	56	6.1*$
SMA (6)	-4	-2	64	6.0*$	4	4	58	5.0*$
	-2	6	52	5.3*$	8	-22	62	4.8*$
	-6	-18	58	5.3*$				
	-6	4	54	5.3*$				
	-6	16	52	4.8*$				
Precentral gyrus (6)	-32	-10	52	5.1*$				
Paracentral lobule (4)	-4	-26	62	5.0*$				
	-4	-32	64	4.8*$				

A. Motor component of the virtual walking task in the Imagery condition (continued)

	Left Hemisphere				Right Hemisphere			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
Postcentral gyrus					30	-28	42	4.8*$
Sup. parietal gyrus (40)					40	-44	62	5.1*$
Rolandic opercular gyrus					54	-26	24	6.1*$
					58	2	8	3.9$
					58	10	10	3.9$
					48	6	12	3.2$
					60	-6	14	3.1$
Precuneus	-2	-38	64	5.1*$				
Sup. temporal pole (38)	-44	20	-16	4.6$				
	-52	14	-14	4.1$				
	-52	-26	12	4.4				
Pallidum					16	4	2	4.3
					14	-2	0	4.1
					18	10	0	3.7
Thalamus					16	-10	4	3.7
					18	-18	0	3.6
					20	-22	0	3.4
					18	-16	4	3.4
					20	-2	10	3.3
					14	-20	2	3.2
					8	-6	-4	3.2
Cerebellum - Vermis					0	-46	-6	4.4$
Cerebellum – III Lobule	-12	-34	-24	6.5*$	16	-32	-24	6.8*$

A. Motor component of the virtual walking task in the Imagery condition (continued)

	Left Hemisphere				Right Hemisphere			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
Cerebellum – IV-V Lobule	-6	-50	-16	5.8*$	10	-42	-18	4.6$
	-28	-44	-30	4.8*$	28	-44	-30	5.6*$
	-26	-54	-26	4.8*$	26	-58	-28	4.7*$
Cerebellum – X Lobule	-18	-34	-40	4.7*$				
Cerebellum – IX Lobule					16	-48	-52	4.5$
					10	-54	-52	3.7$
Cerebellum – VIII Lobule					20	-46	-50	4.1$
					24	-40	-46	3.9$
Brainstem					2	-26	-24	4.4$
					8	-30	-40	4.2$
Precentral gyrus (6/4)					16	-22	76	3.7
Paracentral lobule (4)					10	-22	76	3.7
Cerebellum - Vermis					0	-48	-10	4.6
					2	-56	-10	3.6

x, y, and z are the stereotactic coordinates of the activations in the MNI space.
Statistical threshold $p < .001_{uncorr}$. Only voxels included in significant clusters are reported.
(*) Z-scores statistically significant also after FWE (family-wise error) correction.
($) Voxel included in a cluster surviving the FWE-correction at cluster-level.

B. Motor component of the virtual walking task in the Imitation condition (continued)								
	Left Hemisphere				Right Hemisphere			
Area (BA)	**X**	**Y**	**Z**	**z-score**	**X**	**Y**	**Z**	**z-score**
Cerebellum – IV-V Lobule	-18	-44	-26	4.1				
	-16	-36	-26	4.1				
	-24	-40	-28	3.8				
	-16	-48	-26	3.7				
	-26	-42	-30	3.5				

C. Motor components of the virtual walking task in the Imagery ∩ Imitation condition.								
	Left Hemisphere				Right Hemisphere			
Brain area (BA)	**X**	**Y**	**Z**	**z-score**	**X**	**Y**	**Z**	**z-score**
Cerebellum – Vermis	-2	-48	-8	4.2	0	-48	-12	4.2
Cerebellum – IV-V Lobule	-16	-36	-26	4.1				
	-24	-40	-28	3.8				
	-18	-50	-24	3.4				

x, y, and z are the stereotactic coordinates of the activations in the MNI space.
Statistical threshold $p < .001_{uncorr}$. Only voxels included in significant clusters are reported.
(*) Z-scores statistically significant also after FWE (family-wise error) correction.
($) Voxel included in a cluster surviving the FWE-correction at cluster-level

Table 3.4 a, b Interaction analysis: (a) 'Imagery(visual&motor) > Imagery(visual)' > 'Imitation(visual&motor) > Imitation(visual)' contrast; (b) 'Imitation(visual&motor) > Imitation(visual)' > 'Imagery(Visual&Motor) > Imagery(Visual)' contrast. Virtual Walking analysis.

A. Imagery > Imitation									
		Left Hemisphere				**Right Hemisphere**			
Cluster-size	**Brain area (BA)**	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
N =136	Supramarginal gyrus					54	-24	26	4.3
	Postcentral gyrus					42	-16	30	3.8
						38	-20	36	3.2
N = 92	Cerebellum – III Lobule	-10	-36	-20	3.5				
	Cerebellum – IV-V Lobule	-14	-38	-18	3.3				
	Brainstem (MLR)	-4	-26	-24	3.8	2	-26	-24	3.6
		-6	-32	-24	3.7	6	-24	-22	3.1
		-8	-28	-24	3.7				
B. Imitation > Imagery									
		Left Hemisphere				**Right Hemisphere**			
Cluster-size	**Brain area (BA)**	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
No significant results									

x, y, and z are the stereotactic coordinates of the activations in the MNI space.
Statistical threshold $p < .001_{uncorr}$. Only voxels included in significant clusters are reported.

Table 3.5 Main effect of Feet Movements (Feet Movement analysis, 1 1 1 1 contrast)

Main effect of Feet Movements								
	Left Hemisphere				**Right Hemisphere**			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
Mid. cingulum (24)	-8	2	38	3.6*$				
Mid. cingulum / SMA (24/6)					6	-2	48	4.4*$
SMA / Paracentral lobule (6/4)					8	-18	64	> 8*$
Paracentral lobule (4)	-6	-20	68	> 8*$	6	-28	68	> 8*$
	-6	-30	68	> 8*$				
Rolandic opercuar gyrus					52	-26	20	3.8
					50	-28	22	3.7
Sup. temporal gyrus (42)					56	-24	16	3.8
Putamen					30	-18	12	4.0
Thalamus					22	-16	16	3.4
Cerebellum – Vermis					0	-46	-12	> 8*$
Cerebellum – IV-V Lobule	-14	-36	-24	> 8*$	18	-36	-24	> 8*$
Cerebellum – VI Lobule	-30	-46	-28	3.7$				

x, y, and z are the stereotactic coordinates of the activations in the MNI space.
Statistical threshold $p < .001_{uncorr}$. Only voxels included in significant clusters are reported.

APPENDIX B

Table 4.2 healthy subjects main effects

Imagery analysis: Healthy subjects > Patients **Masked for the simple effect of Walk(-) imagery of the healthy subjects**								
	Left Hemisphere				**Right Hemisphere**			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
putamen					28	8	14	4.01
					30	-12	14	3.84
					26	0	16	3.58
					24	-6	12	3.29

x, y, and z are the stereotactic coordinates of the activations in the MNI space.
Statistical threshold $p < .001_{uncorr}$. Only voxel included in significant cluster are reported.

Table 4.3 visual&motor effect for the healthy subjects over patients (-1 0 1 0)

Imagery analysis visual&motor effect: Healthy subjects > Patients (continued)

Masked for the simple effect of Walk(-) imagery of the healthy subjects

	Left Hemisphere				Right Hemisphere			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
cingulum mid(32)	0	22	38	3.36$	8	18	44	3.85$
SMA (6)	-8	10	50	4.28$	6	2	58	3.23$
parietal sup (7)	-28	-62	56	3.95$	22	-50	54	4.24
	-26	-60	52	3.94$				
	-28	-56	50	3.94$				
	-16	-62	54	3.83$				
parietal inf (40)	-30	-52	48	3.94$				
	-30	-48	46	4.02$				
	-38	-44	44	3.97$				
precuneus (5)					8	-48	60	3.65$
					14	-44	52	3.54$
					22	-66	20	3.34$
cuneus (19)	-8	-84	30	3.45				
	-14	-84	30	3.25				
temporal mid (37)					46	-50	-2	3.66
					44	-54	12	3.63
occipital sup (19)	-22	-70	24	3.98	24	-66	32	3.73
	-24	-64	28	3.62	22	-64	36	3.65

Imagery analysis visual&motor effect: Healthy subjects > Patients (continued)

Masked for the simple effect of Walk(-) imagery of the healthy subjects

	Left Hemisphere				Right Hemisphere			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
occipital mid (18)	-28	-88	2	3.68				
	-32	-78	0	3.67				
	-32	-88	10	3.56				
putamen					22	-8	10	3.41$
					20	10	8	3.33$
					28	8	14	3.26$
					24	4	12	3.24$
					26	-10	12	3.41$
					20	10	2	3.56$
					34	2	8	3.51$
					28	6	10	3.48$
					20	-4	12	3.44$
					28	-2	18	4.07$
					30	-14	14	3.67$
brainstem	-10	-30	-26	4.12				
	-8	-34	-24	3.99				
	-8	-28	-36	3.33				
cerebellum 6 l	-22	-72	20	4.10				

x, y, and z are the stereotactic coordinates of the activations in the MNI space.
Statistical threshold $p < .001_{uncorr}$. Only voxel included in significant cluster are reported.
(*) Z-scores statistically significant also after the FWE (family-wise error) correction.
($) Voxel included in a cluster surviving the FWE-correction at cluster-level.

Table 4.4 interaction effect for the imagery task evidencing the visual&motor component for the patients group

Imagery task: Interaction effect *'visual > visual&motor' x 'patients > healthy subjects'(-1 1 1 -1)*								
Brain area (BA)	**Left hemisphere**			**z-score**	**Right hemisphere**			**z-score**
postcentral (3)					40	-18	34	4.14$
					34	-28	38	4.10$
	-58	-12	32	3.99	40	-24	46	3.26$
superior parietal lobule (7)	-28	-60	54	4.20$				
parietal inf (40)	-30	-50	34	3.33$	36	-44	38	3.25$
	-34	-50	40	3.32$				
	-32	-48	50	3.30$				
	-40	-40	38	3.28$				
	-28	-46	46	3.27$				
	-36	-48	44	3.16$				
	-38	-44	38	3.15$				
temporal sup	-44	-26	8	3.99				
lingual (27)	-10	-38	0	4.36$				
occipital mid (19)	-34	-76	20	4.03				
	-32	-78	10	3.29				
	-30	-78	6	3.24				
calcarine (18)	-10	-68	18	3.67				
thalamus	-16	-24	4	3.34$				
hippocampus	-24	-28	0	3.57$				
vermis					0	-42	-4	4.02$

Imagery task: Interaction effect (continued)

'visual > visual&motor' x 'patients > healthy subjects'(-1 1 1 -1)

Brain area (BA)	Left hemisphere			z-score	Right hemisphere			z-score
cerebellum	-12	-40	-14	3.56				
	-10	-36	-20	3.33				
	-12	-32	-38	3.18				
brainstem	-8	-28	-24	4.27	2	-26	-24	3.76
	-8	-28	-36	3.46	10	-28	-24	3.69
					6	-32	-24	3.19
Corpus callosum	-22	-72	18	4.07				
	-14	-70	18	3.87				
Occipito frontal fasciculus	-38	-22	-2	3.52				
	-38	-16	-2	3.22				

x, y, and z are the stereotactic coordinates of the activations in the MNI space.
Statistical threshold $p < .001_{uncorr}$. Only voxel included in significant cluster are reported.
(*) Z-scores statistically significant also after the FWE (family-wise error) correction.
($) Voxel included in a cluster surviving the FWE-correction at cluster-level.

Table 4.5 visual&motor effect for the healthy subjects group in the imitation condition

Imitation analysis visual&motor effect: Healthy subjects > Patients **Masked for the simple effect of Walk(-) imagery of the healthy subjects**								
	Left Hemisphere				**Right Hemisphere**			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
paracentral lobule (4,6)	-6	-16	66	3.70$	0	-26	72	3.66$
parietal sup (7)	-26	-54	64	3.98$				
	-24	-62	50	3.48$				
	-20	-60	48	3.38$				
parietal inf (40)	-30	-44	44	3.56$				
	-26	-42	40	3.42$				
	-32	-44	38	3.31$				
	-36	-46	52	3.23$				

x, y, and z are the stereotactic coordinates of the activations in the MNI space.
Statistical threshold $p < .001_{uncorr}$. Only voxel included in significant cluster are reported.
(*) Z-scores statistically significant also after the FWE (family-wise error) correction.
($) Voxel included in a cluster surviving the FWE-correction at cluster-level.

Table 4.6 interaction analysis for the visual over the visual&motor component in the patients group

Imitation analysis interaction effect

'visual > visual&motor' x 'patients > healthy subjects'(-1 1 1 -1)

	Left Hemisphere				Right Hemisphere			
Brain area (BA)	**X**	**Y**	**Z**	**Z-score**	**X**	**Y**	**Z**	**Z-score**
cingulum	-24	-46	-8	3.81$				
lingual	-26	-42	-2	4.11$				
Retrosplenial corpus callosum	-14	-38	22	4.88				
	-24	-32	24	4.24				
Occipito frontal fasciculus	-32	-50	-12	3.42$				
Optic radiation	-34	-46	2	3.89$				
Arcuate posterior segment	-54	-44	20	3.55$				
	-38	-48	8	4.96$				

x, y, and z are the stereotactic coordinates of the activations in the MNI space.
Statistical threshold $p < .001_{uncorr}$. Only voxel included in significant cluster are reported.
(*) Z-scores statistically significant also after the FWE (family-wise error) correction.
($) Voxel included in a cluster surviving the FWE-correction at cluster-level.