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Ph.D. Course: Neuroscience – Motor and Sport Activities

Ph.D. Course: Sciences and Techniques of Physical and Sporting Activities

Neural Contributions to Physical Activity: From the Brain to the Muscle and Back Again

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ABOUT THIS WORK

This thesis results from three years of in-depth study and research conducted in collaboration with the University of Burgundy. The thesis aims to explore the neural contribution to physical activity. The work is divided into two chapters containing research conducted in Italy, France or both states. The first chapter includes four behavioural studies aimed at evaluating the reciprocal relationship between physical activity and cognition. The second chapter contains two neurophysiological studies aimed at investigating the muscle length and the type of contraction's contribution to the activity of the motor system.

Chapter one investigates how motor condition influences cognitive processes and vice versa. Several studies suggested that engaging in physical activity programs elicits a wide range of neural changes. One of those is enhancing cognitive performance (e.g., working memory or information processing speed). On the other side, specific cognitive interventions (e.g., action observation or motor imagery) can ameliorate motor performance. This reciprocal interaction could produce adverse effects if people exceed one of these two activities, which induce a fatiguing state. Before the studies' exposition, cause the variety of topics treated in chapter one, it will be discussed three issues providing a general view of:

- the benefit of physical activity in mental and brain health
- the adverse consequence of a muscle and mental fatigue state
- the learning effect induced by action observation and motor imagery

After the elaboration of these issues, it will be presented four behavioural studies, which investigated the four interactions between physical and mental activity.

The first study checked the correlation between physical exercise and executive performance in forty subjects (middle, high school, and university students), assessing a good motor condition's positive effects on working memory functions.

The second study aimed to verify whether a cognitive practice such as motor imagery could positively affect the performance of a motor gesture. Also, in this case, subjects belonging to the school group were tested, specifically high school students.

The third study examined the possible adverse effects that excessive motor practice (muscle fatigue) could bring on a cognitive level. Precisely, it was verified whether a condition of muscle fatigue could alter a subject's capacity for temporal expectation.

The fourth study investigated the possible adverse effects of excessive cognitive practice (mental fatigue) on a motor level. It verified whether a continuous and repeated action observation could alter the subject's motor performance.

Chapter two looks into the activity of the corticomotor system as a function of muscle length and type of contraction. After a general introduction exploring previous studies investigating the role of muscle length (in static and dynamic form) on the corticomotor system, the chapter will present two studies.

The first study examined the influence of muscle length on neuromuscular function and corticospinal excitability.

The second study investigated the difference in primary motor cortex excitability when preparing concentric and eccentric contractions.

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CHAPTER 1

1.1 Physical activity and cognition: How motor condition influences cognitive processes and vice versa

The *World Health Organization* (WHO) defines physical activity as any bodily movement produced by skeletal muscles that require energy expenditure. It refers to all movements, including leisure time, transport to get to and from places, or part of a person's work.

Physical activity has been widely indicated as a strategy for promoting health in all ranged population, from infants (less than one year) to the elderly (above 65). Studies suggest that the benefits induced by regular physical activity reach both aspects of human beings: body and mind (5, 32).

More than 60 years ago, Morris and colleagues (29) conducted one of the first work investigating the importance of physical activity in health benefit. They noted that men in physically active jobs (i.e., postal workers) had a lower incidence of coronary heart disease in middle age than men in physically inactive jobs (i.e., transport workers). More importantly, the disease in physically active workers was not so severe, tending to present first in them as angina pectoris and other relatively benign forms and to have a smaller early case fatality and a lower early mortality rate. This research is the pioneer study showing the importance of physical activity in preventing pathologies related to a sedentary lifestyle.

A series of studies had investigated the potential benefit associated with physical activity in several diseases in the years to come.

In 1990 Cochrane and Clark (8) tested the clinical and physiological effects of a medically supervised, indoor physical training programme in 36 asthmatic subjects, obtaining significant improvements in fitness and cardiorespiratory performance.

In 1991 Eriksson and Lindgärde (13) screened 6956 men with type 2 diabetes mellitus and planned for 41 of them a physical activity program that produced substantial metabolic improvement and reduced the symptoms related to the disease.

Years later, a series of studies had accumulated evidence linking physical exercise and a wide variety of other chronic diseases, such as cancer (colon and breast), obesity, and bone and joint diseases (osteoporosis and osteoarthritis) (40).

Nowadays, physical exercise is a primary prevention factor to reduce mortality risk (Figure 1.1.1) (1, 43).

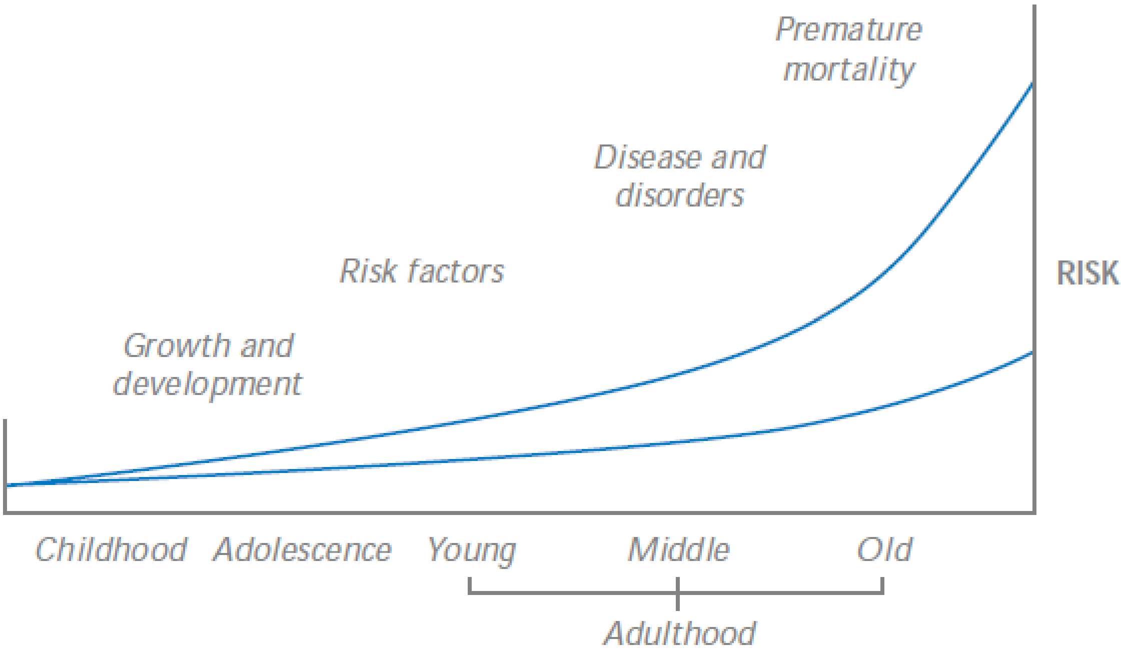

Figure 1.1.1 | Physical activity and disease/mortality risk in a lifetime perspective: the lower line shows the hypothetical risk in active subjects, the upper line in inactive subjects (from Department of Health, 2004 and published from Zaccagni et al. 2014 (43)).

For this reason, WHO widely recommended constant motor activity (about 30 minutes/die of moderate intensity at least five days a week), which should be considered part of primary health care (19, 21). This amount of activity can be achieved in any number of ways during leisure time and at work, with the required duration depending on relative intensities of the activities undertaken (Figure 1.1.2) (28).

Engaging in physical activity, in addition to the physical benefits described above, can also contribute to mental and brain health. Exercise improves emotional well-being, reduces symptoms of depression and anxiety (34), and brings positive advantages in neurophysiological disease (30) and mental disorders (15).

Regular exercise also contributes to brain health, promoting a wide range of neural changes. In rodent (38), voluntary exercise alone is sufficient for enhanced neurogenesis. In healthy humans, short-term exercise increases the circulating brain-derived neurotrophic factor (BDNF) level (9), a protein that facilitates neurogenesis and neuroprotection (36).

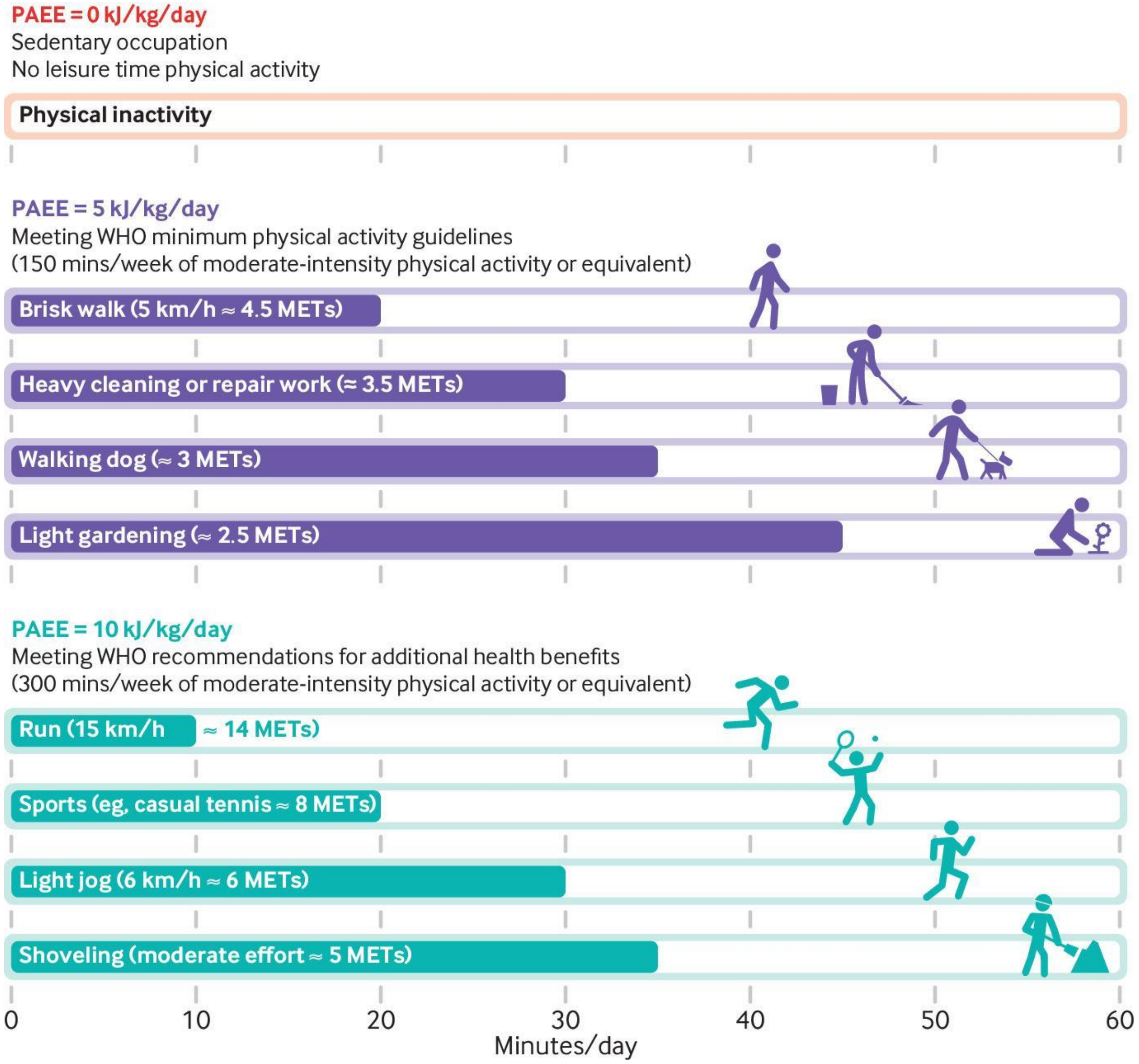

Figure 1.1.2 | Physical activity energy expenditure (PAEE) of common activities performed during leisure time and at work. MET=metabolic equivalent of task. WHO=World Health Organization (from Mok et al. 2019).

Physical activity also elicits structural modifications in the human brain. Voss et al. (40) observed that greater aerobic fitness was associated with significant changes in white matter integrity in the frontal and temporal lobes in older adults. Other studies reported evidence of a protective (or even restorative) role of cardiorespiratory fitness against cognitive and neurobiological impairments (24, 25, 40).

Finally, aerobic exercise induces effects at a behavioural level producing outcome in cognitive function. For example, Voss et al. (40) showed that individual differences in aerobic fitness were associated with cognitive control performance among children. A few years later, Kao et al. (22) observed in children a significant positive correlation between aerobic fitness and working memory as well as aerobic fitness and academic achievement. These mental and brain benefits associated with physical activity prompted researchers to investigate the topic linking physical activity and cognition profoundly.

The term cognition, referred to academic or general cognitive performances, is a mental function involved in learning and comprehension (14).

In one way, it was showed that engaging in physical activity programs elicit a wide range of neural changes (12). One of those is improving executive functions associated with executive control processes, such as planning, scheduling, inhibition, and working memory (23).

On the other way, recent studies also established that several types of cognitive or combined cognitive-motor intervention might improve physical functions (32) and motor performances (4, 18). For example, motor imagery (the mental simulation of action without its actual execution (20)) and action observation (the process of observing actions performed by other people) are two mental techniques that were successfully applied as motor learning techniques (2–4, 11, 36).

The scientific rationale behind this idea is that motor imagery and action observation activate neural substrates partially overlapped with those activated by movement execution (Figure 1.1.3) (16, 20, 34). A shared neural representation would support the hypothesis that motor imagery and action observation may promote neural plastic changes and behavioural improvements similar to movement execution.

In the half of the nineties, Pascual-Leone (31) discovered that enhancement in motor performance could be obtained with physical and mental practice. In 15 subjects, he used motor imagery as a mental activity to produce positive effects in learning new piano sequences.

When associated with somatosensory input, motor imagery influences motor performance similarly to motor execution, increasing movement speed, inducing plasticity, and retaining motor skill in the following days (7).

Similarly, action observation facilitates motor planning, execution, and memory formation. The athletes' training frequently includes the attentive viewing of sport and other performances by applying the same concepts of motor imagery described above.

Unfortunately, this reciprocal influence between mental and physical activity does not produce exclusively positive effects. As research has shown, the impact of motor exercise on executive function could be harmful when people overcome a specific motor effort and approach exhaustion (10), which disrupts the mental benefits related to exercise and promote an inhibiting effect on cognitive performance (26).

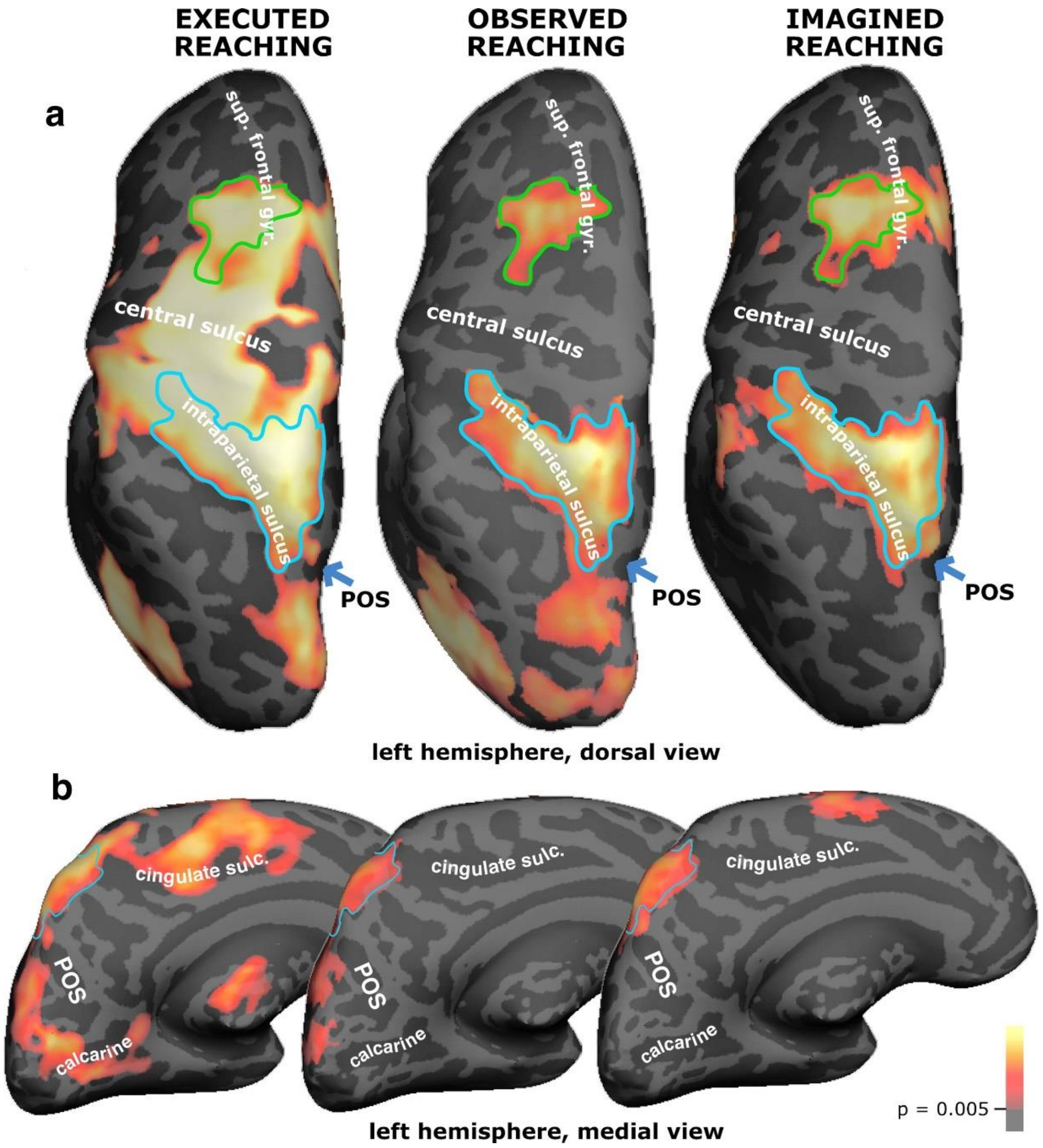

Figure 1.1.3 | Outline of overlap between executed, observed, and imagined reaching in left dorsal premotor (superior frontal sulcus and gyrus) and left posterior parietal areas, on group surface-averaged activations from 15 subjects, displayed on one subject's inflated hemisphere. (a) Dorsal view of left hemisphere. (b) Medial view of left hemisphere. Executed, observed, and imagined reaching all activated a medial parietal area located in-between the parieto-occipital sulcus and the posterior end of the cingulate sulcus, outlined in light blue. Sup. frontal gyr = superior frontal gyrus; POS = parieto-occipital sulcus; calcarine=calcarine sulcus; cingulate sulc = cingulate sulcus. From Filimon et al. 2007 (16).

Similarly, also reciprocal interaction was observed. Marcora et al. (27) demonstrated that physical performance results in impairment after a prolonged cognitive task (more than 30 minutes). He found that mentally fatigued people reached their maximal level of perceived exertion and disengaged from a physical task earlier than people mentally rested.

Summarising the concepts described above, we can assume that taking part in controlled and well-dosed physical or mental activity programs can improve the reciprocal activity without directly training it. However, if people exceed one of these two activities (physical exercise or mental activity), they could negatively affect them.

These contrasting results obtained are discriminated by the presence or not of fatigue.

Fatigue can refer to a subjective symptom of malaise and aversion to activity or to objectively impaired performance. It has physical and mental aspects (38), which take muscle and mental fatigue names. Muscle fatigue is an exercise-induced reduction in maximal voluntary force (17), while mental fatigue represents a psychobiological state caused by prolonged periods of demanding cognitive activity (6).

After an elaboration of several topics concerning the key concepts of physical activity in health, fatigue, action observation and motor imagery, this chapter will present four studies, which investigated the four interactions between physical and mental activity described above.

The first study checked the correlation between physical exercise and executive performance in forty subjects (middle, high school, and university students), assessing a good motor condition's positive effects on working memory functions.

The second study aimed to verify whether a cognitive practice such as motor imagery could positively affect the performance of a motor gesture. Also, in this case, subjects belonging to the school group were tested, specifically high school students.

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1.1.1 Physical activity in mental and brain health

The oldest studies linking physical activity and mental health investigated the role of physical exercise in the management of mild-to-moderate mental health diseases, especially depression and anxiety (27, 28).

Physical exercise was primarily associated with psychological well-being because it was shown to increases levels of endorphins (8, 33), endogenous opioid polypeptides compound produced during strenuous exercise, excitement and pain.

Physical exercise that overlaps a certain threshold of workload produce a neurophysiologic sense of well-being (9) because induces an increased exertion of norepinephrine (8). In addition, exercise's benefits well-accepted include also psychologic well-being through improved self-esteem, feelings of achievement and accomplishment, improved body image, and stress reduction (28).

The beneficial effects of physical activity and exercise on depression symptoms and general mood have been confirmed in individuals of all ages. A small beneficial effect of exercise in reducing depression and anxiety scores was observed in children and adolescents (25). In younger adults, exercise interventions increase positive mood (14), while in older adults alleviate symptoms of major depression (16, 26). Elderly who remains physically active across time demonstrate lower levels of depression symptoms compared to those who adopt inactive lifestyles when they get older (24).

Regular exercise also contributes to brain health, promoting a wide range of neural changes.

In 1999, Praag (36) exposed rodents to various conditions: water-maze learning (learner), swim-time-yoked control (swimmer), voluntary wheel running (runner), and enriched (enriched) and standard housing (control) groups. This study determined whether physical activity, whether voluntary, forced or combined with a learning task, was involved in the enhanced adult mice hippocampal neurogenesis, just previously observed in the late nineties following exposure to enriched environments (21, 22). The results showed that cell proliferation increased in mice housed with unrestricted access to a running wheel (Figure 1.1.1.1) (runners).

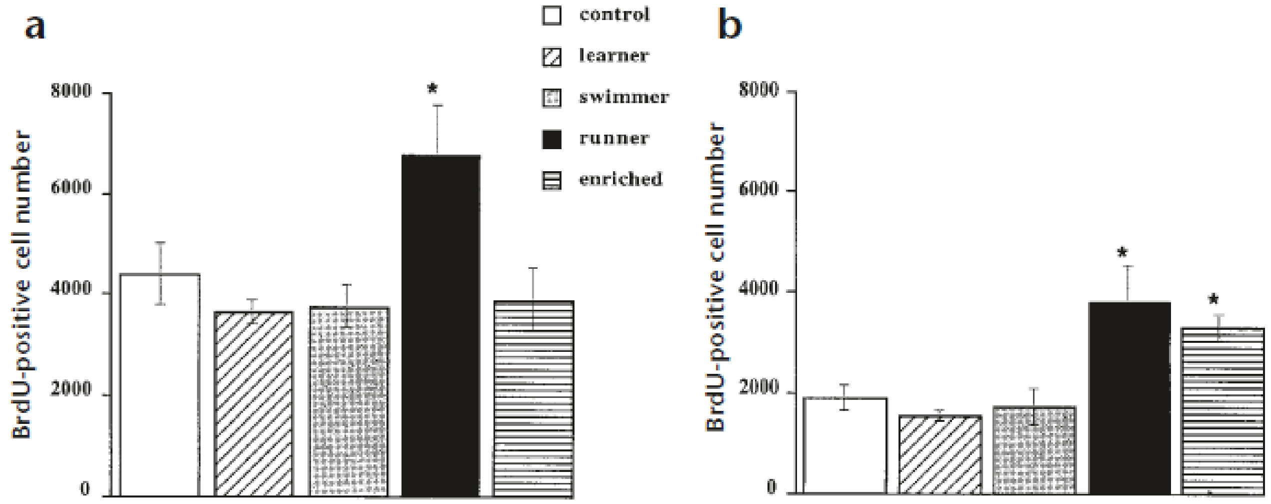


Figure 1.1.1.1 | BrdU-positive cell number. (a) Total number of BrdU-positive cells per dentate gyrus one day after the last BrdU injection, to estimate ongoing proliferation. Significantly more cells were labelled in the runners as compared to the other groups. $*p < 0.02$. (b) Total number of BrdU-positive cells per dentate gyrus four weeks after the last BrdU injection, to estimate survival of labelled cells. Enrichment and running significantly increased the survival of newborn cells. $*p < 0.02$. From Praag et al. 1999 (36).

This potential neurogenesis induced by training was attributed to different proteins capable of favourite the brain's growth. At present, brain-derived neurotrophic factor (BDNF), insulin-like growth factors (IGFs) and vascular endothelial-derived growth factor (VEGF) are the principal growth factors known to mediate the effects of exercise on the brain. These growth factors work in concert to produce complementary functional effects, modulating both overlapping and unique aspects of exercise-related benefits in brain plasticity, function, and health (7).

Current literature clearly shows that physical activity enhances BDNF serum levels but reported results slightly discordant related to IGF-1.

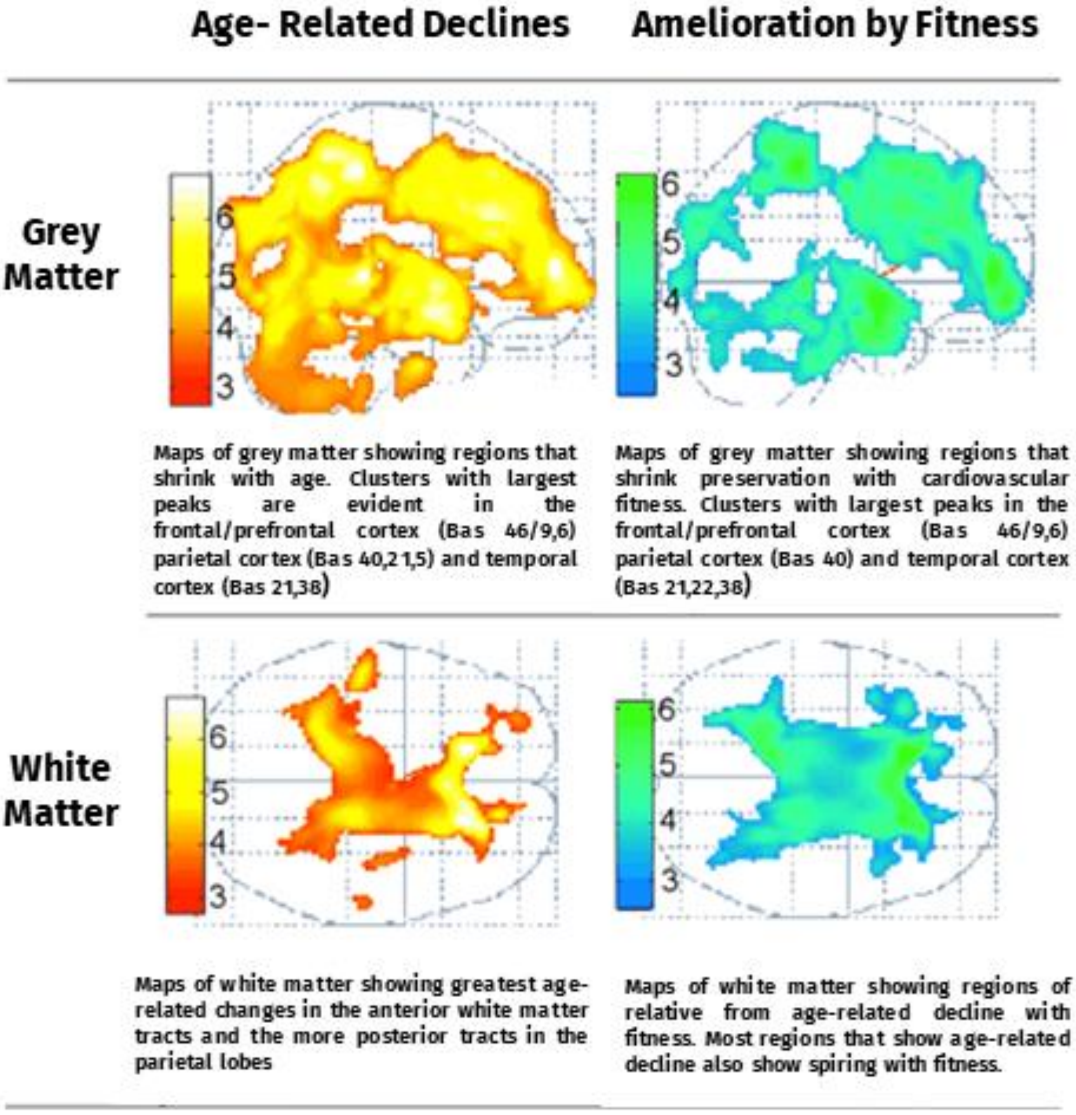
Recently, Griffin and colleagues (15) demonstrated that a short period of high-intensity cycling results in enhancements of concentration of BDNF, but not IGF-1, in the serum of exercising adults. If the aerobic training is maintained for five weeks, the serum BDNF response to acute exercise increase.

A few years later, Jeon and Ha (18) enrolled 20 junior-high-school students with no history of physical illness and divided them into an exercise group and a control group. The exercise group performed treadmill exercise (consuming 200 kcal) three times per week for eight weeks. The exercise group showed statistically significant in increases serum BDNF and IGF-1 after eight weeks, suggesting that long-term regular aerobic exercise positively affects serum BDNF levels at rest and IGF-1 of adolescents (who are still undergoing brain developments).

Meanwhile, research on children and young adults concentrated on exercise's neurogenetic property; studies on older adults investigated the effects of aerobic exercise in grey and white matter volumes.

Ageing is often characterised by deterioration of both white matter and grey matter tissues in the prefrontal, temporal, and parietal cortices with relative sparing of tissue in other regions such as the primary motor and visual cortex. For this reason, the effect of exercise on grey and white matter volumes was firstly investigated in older adults' populations.

Colcombe et al. (5) demonstrated, in a cross-sectional sample of fitter and less fit older adults, that fitter individuals had greater grey matter volume in the prefrontal, parietal, and temporal regions and greater white matter volume in the genu of the corpus callosum than their less-fit counterparts (Figure 1.1.1.2).



Maps of grey matter showing regions that shrink with age. Clusters with largest peaks are evident in the frontal/prefrontal cortex (Bas 46/9,6) parietal cortex (Bas 40,21,5) and temporal cortex (Bas 21,38)

Maps of grey matter showing regions that shrink preservation with cardiovascular fitness. Clusters with largest peaks in the frontal/prefrontal cortex (Bas 46/9,6) parietal cortex (Bas 40) and temporal cortex (Bas 21,22,38)

Maps of white matter showing greatest age-related changes in the anterior white matter tracts and the more posterior tracts in the parietal lobes

Maps of white matter showing regions of relative from age-related decline with fitness. Most regions that show age-related decline also show spiring with fitness.

Figure 1.1.1.2 | Statistical maps derived from multiple regressions of age and cardiovascular fitness on grey (top row) and white matter (bottom row) density. Images are rendered for display purposes as glass brains, thresholded at a minimum Z of 3.25, p, .002, collapsing across the sagittal (top row) and axial (bottom row) planes. The brighter colours represent greater tissue density changes with age (left side) and greater sparing of tissue density with increasing fitness (right side). From Colcombe et al. 2003 (5).

A few years later, the same group of authors (6) found that the six months of aerobic training increased grey matter volume in the frontal and superior temporal lobe and increased white matter volume in the genu of the corpus callosum, while the control group (who participated to toning and stretching training) underwent a slight decline in cortical volume. These results suggested that cardiovascular fitness is associated with the sparing of brain tissue in ageing humans and that even relatively short exercise interventions can begin to restore some of the losses in brain volume associated with normal ageing.

The training time of six months could be the closest capable of producing significant effects in white and grey matter structures. Indeed, a recent study (30), investigating the effects of a shorter aerobic training programme (twelve-week) on cardiorespiratory fitness and magnetic resonance imaging (MRI) outcomes, found an increased cardiorespiratory fitness which does not produce significant changes in measures of brain structure in older adults.

Erickson and colleagues (10) carried out a randomised controlled study with 120 older adults participating in aerobic exercise or stretching and toning condition (control) lasting one year. He observed that aerobic exercise training increases the size of the anterior hippocampal volume by 2% and associated this finding with greater serum levels of BDNF. All these studies obtained a significant increase in white or grey matter volumes applying to participants an aerobic exercise program, while other physical activity programs obtain inconsistent results.

Physical activity and aerobic fitness are distinct concepts. Caspersen (3) defined physical activity as "any bodily movement produced by skeletal muscles that result in energy expenditure". On the other hand, physical fitness is a condition or adaptive state that individuals have or achieve.

Ruotsalainen et al. (29) measured both physical activity and aerobic fitness in the same study to differentiate the association between these two measures in grey matter volume. This study, conducted on sixty adolescents, demonstrated the unequal contribution of physical activity and aerobic fitness on grey matter volumes, with inherent or achieved capacity (aerobic fitness) showing more evident associations than physical activity.

These improvements in grey matter volume described above are accompanied by altered brain activity measures, translating into a more efficient and effective neural system. For this reason, some of the studies previously exposed, in addition to white and grey matter volumes, also measured memory, processing speed and other executive functions (10, 15, 30).

Recent reviews concluded that exist a positive relationship between physical activity and cognitive functioning in all range populations (2, 11, 12, 32). A series of tools were used to assess cognitive functioning, such as perceptual skills, intelligence quotient, achievement, verbal tests, math tests, memory, developmental level/academic readiness, creativity, concentration, cross-disciplinary batteries, and others. Working memory and perceptual skills are two of the cognitive functions most studied in association with physical exercise. This could be owed to their easy-administration tests, reliability and validity of assessments, or their role in cognitive development and predictiveness of real-world outcomes (34).

Perceptual speed reflects the ability to compare different stimuli accurately and quickly (e.g., numbers, symbols, patterns) while working memory reflects the capacity to maintain information in active memory while simultaneously performing distracting or interfering activities (34).

Sibley's meta-analysis (32) determined a positive relationship between physical activity and cognitive performance in school-age children (aged 4–18 years) in eight measurement categories (perceptual skills, intelligence quotient, achievement, verbal tests, mathematic tests, memory, developmental level/academic readiness and other). A beneficial relationship was found for all categories, except for memory, which was unrelated to physical activity behaviour, and for all age groups (although it was stronger for children in the age ranges of 4–7 and 11–13 years, compared with the age ranges of 8–10 and 14–18 years).

Until 2011 most studies involving children have focused on the relationship between aerobic fitness and academic achievements rather than correlate it to a subset of top-down mental processes which implement goal-directed behaviour and enhance academic performances (4, 12, 17).

Kamijo and colleagues (19) firstly observed several improvements in preadolescent's working memory after a 9-month physical activity intervention.

Subsequently, Kao et al. (20) investigated the relationship between working memory and academic achievement with aerobic and muscular fitness. Hierarchical regression analyses indicated that after controlling for demographic variables (age, sex, grade, IQ, socioeconomic status), higher aerobic and muscular fitness levels were associated with greater performance in task conditions that placed greater demand on working memory. By contrast, only aerobic fitness showed a positive correlation with mathematic performance in algebraic functions, suggesting a selective benefit related to cortical grey matter thinning during brain maturation.

In young adults (aged 19-30), a similar association was observed. Lambourne (23) recruited 42 male and female college students divided into groups based on self-reported physical activity level. The physically active group contained participants who met the physical activity requirements specified by the Center for Disease Control and Prevention, while the inactive group included only inactive people. Both groups performed a reading span task to assess the participant's working memory capacity. Analysis of variance results demonstrated that exercise was associated with enhanced working memory, generalising previous results obtained in young people.

More objective measurement still referred to young adults were collected in a study by Åberga (1). This research, conducted in larger sample size, aimed to determine the association between physical activity and cognitive performance, screening results for military service at 18 years of age from all Swedish men born from 1950 through 1976. He correlated test's results from more than one million men enlisted for military service. When were recruited, the freshmen performed a cycle-ergometry test (to assess cardiorespiratory fitness), a series of isometric muscle strength tests, and four cognitive tests covering the following areas: logical performance test, a verbal test of synonyms and opposites, a test of visuospatial/geometric perception, and technical/mechanical skills including mathematical/physics problems. The results showed that cardiovascular fitness, but not muscular strength, was positively associated with intelligence after adjusting for relevant confounders (Figure 1.1.1.3).

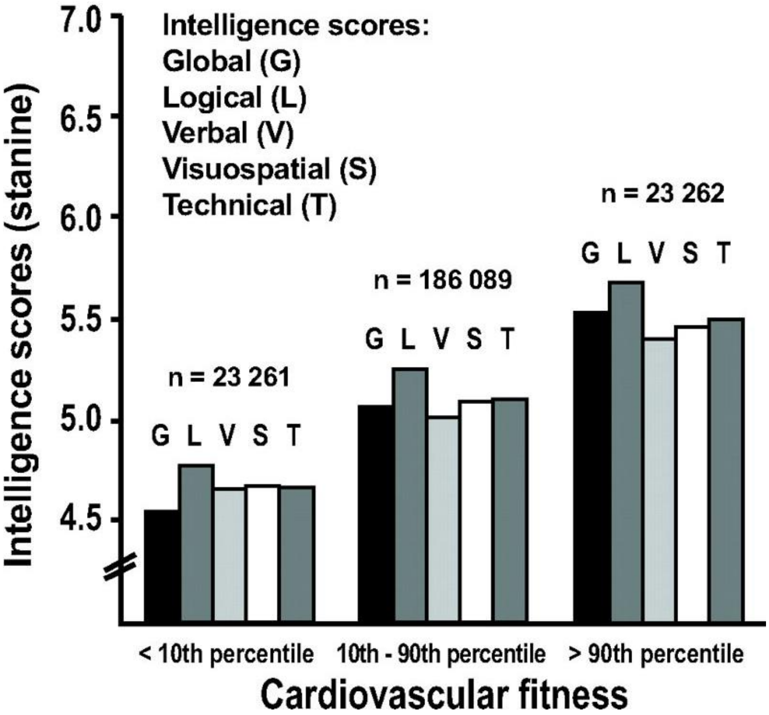


Figure. 1.1.1.3 | Change in cardiovascular fitness between age 15 y and 18 y predicts intelligence scores. From all subjects with physical education grades at age 15 y and cardiovascular fitness scores at age 18 y, the 10% of subjects with the highest and lowest changes in fitness scores compared with predicted scores were selected (<10th percentile; 10% lowest vs. predicted scores; > 90th percentile, 10% highest vs. predicted scores; 10th-90th percentile, remaining 80%). Mean global intelligence, logical, verbal, visuospatial, and technical scores were compared among the 3 percentile groups and significant differences were found among all groups ($P < 0.0001$). The SDs were 1.81–1.97. From Åberga et al. 2009 (1).

Similar results were later obtained by Esmaeilzadeh et al. (11). In this research, conducted in larger sample size, authors aimed to explore the association of different components of physical fitness (i.e. aerobic fitness and muscular strength) and motor fitness (i.e., speed and agility) with cognition (processing speed and inhibition) in a sample of 211 young males (aged 19-24). He found, also after adjustment for potential confounders (e.g. age, socioeconomic status, adiposity and physical activity), that aerobic fitness, represented by shorter time in the one-mile run, was positively associated with composite inhibitory control scores. In contrast, explosive strength was negatively associated with composite information processing scores and composite inhibitory control scores. This study, also considering previous research, confirmed that cardiorespiratory fitness is an essential aspect of physical fitness to indicate the level of cognitive performance.

In the same year, a group of the University of Dallas screened the older adults' population (13). The research group measured physical function such as cardiorespiratory fitness and cognitive function tests in about five thousand men and women aged 55 and older. Cardiorespiratory fitness was quantified as the duration of a maximal treadmill exercise test highly correlated with laboratory measures of maximal oxygen uptake in men and women. Cognitive function was assessed via the MoCA, a paper and pencil screening tool designed to identify cognitive impairment by assessing multiple cognitive domains, including attention, concentration, executive functions, memory, language, visuospatial skills, calculation, and orientation. Ferrall and colleagues found that low fit elderly (included in quintile 1) had a significantly increased risk of developing cognitive impairment.

Other previous studies showed that exercise training is beneficial for older adults' cognitive functions. For example, Suzuki et al. (31) examined the effects of a 1-year multicomponent exercise training program on the cognitive function of 50 older men and women with mild cognitive impairment. Subjects completed 90 min of supervised aerobic, strength, and balance exercises per day on two days per week. Compared to the control group, who received education only, the exercise intervention group showed significant improvement in general cognitive function, immediate memory, and language ability.

Smith et al. (35) performed a meta-analytic review of randomised control trials that examined the effects of aerobic exercise training on neurocognitive performance. Twenty-nine studies representing 2,049 participants were evaluated. Among individuals randomly assigned to aerobic exercise training, a modest improvement in attention and processing speed, executive function, and memory was found.

Considering all research presented in this paragraph, we can assume that physical activity is associated with improved mood state and induces a wide range of neural changes: from molecular to behavioural levels. This evidence could be considered as one the consequence of the other like physical exercise activates a ripple effect which interest first a molecular change (BDNF and IGF-1), then a structural change (white and grey matter volumes) and at least a behavioural change (improvement or preservation of cognitive functions). Results also suggested that all ranged population could benefit from controlled and well-dosed physical activity programs, with specific age-related positive advantages on cognitive function. The strongest documentations are reported from studies that investigate cardiorespiratory fitness as a measure identifying physical activity level. This last one presents a robust correlation with cognitive functions when estimated through objective measures rather than questionaries.

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1.1.2 Muscle and mental fatigue

The term ***fatigue*** is defined as “an extreme tiredness resulting from mental or physical exertion or illness". This definition opens the possibility of using this term to indicate physiological or pathological conditions as well mental or physical states. In all cases, fatigue is never considered a good state in which people can get.

Firstly, fatigue was studied as a condition interconnected to physical exertion.

The oldest witness on the injurious effects induced by a fatigue condition comes from Arthur Thomson in the far 1800s (66). In 1836 soldiers enrolled in the 17th regiment landed at Bombay from New South Wales split into two groups. One group took part in an exhausting expedition lasting about fourteen months in which soldiers covered distance of 1800 miles and suffered many privations. The other group remained in defence of the regiment. When the two groups have reunited the health of all men was good. Unfortunately, during homecoming, the regiment registered a considerable number of soldiers affected by a pulmonic disease, a circumstance presumed to have been produced by the rapid change of climate and temperature. Thomson noted that soldiers who faced the expedition developed sever symptoms that caused a higher death's number than symptoms developed by soldiers who had not served. He attributed this great difference in the amount of mortality to the exhaustion of the constitution produced by the prolonged and severe exertion to which soldiers engaging the expedition were exposed.

Other studies in the following years investigated fatigue only as a general physical symptom affecting the human body unescorted by direct measures. Only one hundred years later Borg (12) noticed the need for scientists to use a standard method to quantify symptoms related to subjective perceived exertion. He developed a ratio-scaling method to indicate the degree of physical strain with verbal anchors, which partially overcame interindividual comparisons. The Borg's rate of perceived exertion (RPE) scale became very popular and was translated into many languages (Table 1.1.2.1). This scale, currently still used, ranges from 6 to 20 and can be used to denote heart rates ranging from 60 to 200 beats per minute.

A new category scale with ratio properties was developed a few years later (13). The main idea was that numbers had been anchored by verbal expressions easily understandable by most people.

Table 1.1.2.1 | The 15-grade scale for ratings of perceived exertion, the RPE scale from Borg 1970 (12).

6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very Hard
18	
19	Very, very hard
20	

Muscle fatigue

Concomitant to these studies, in the early 1900s, Marx (55) focused his research on the muscle tissue, providing more specific investigations to what is now called muscle fatigue. He studied the chemical products resulting from the muscular activity, such as lactic acid, creatinine, and phosphoric acid. He assumed that the substances generated by muscle activity overflow into the general circulation and, as consequence, produce symptoms of general fatigue. The first effect supposed was the depression of other muscles, including the muscles of the heart and blood vessels. This, in turn, would lead to a diminution of circulation and oxidation and the vicious cycle of added fatigue. He also assumed an altered functioning of the nerve cells and the central nervous system, which would suffer directly from the fatigue substances and indirectly by diminished oxidation. This process finally ended in impairments of muscular power, tempo and coordination of intentional and unintentional motion, and motion sequences. All impairments gradually investigated over the years.

The decline in muscle performance could not interest the muscle's power output exclusively. Indeed, the power output is the product of muscle's force generated and muscle's shortening velocity.

Although both topics were studied, recent research described muscle fatigue as the gradual decrease in muscle force capacity or the endpoint of sustained activity (18). A more accurate, although more general, definition came from Allen and colleagues (1) who described muscle fatigue as any decline in muscle performance associated with muscle activity. However, because fatigue in these definitions can encompass several phenomena that are each the consequence of different physiological mechanisms (18), today most investigators invoke a more focused definition of muscle fatigue: an exercise-induced reduction in the ability of a muscle to produce force or power whether or not the task can be sustained.

A critical feature of this last definition is the distinction between muscle fatigue and the ability to continue the task. Accordingly, muscle fatigue is not the point of task failure or the moment when the muscles become exhausted. Instead, muscle fatigue decreases the maximal force or power that the involved muscles can produce, and it develops gradually soon after the onset of sustained physical activity (Figure 1.1.2.1).

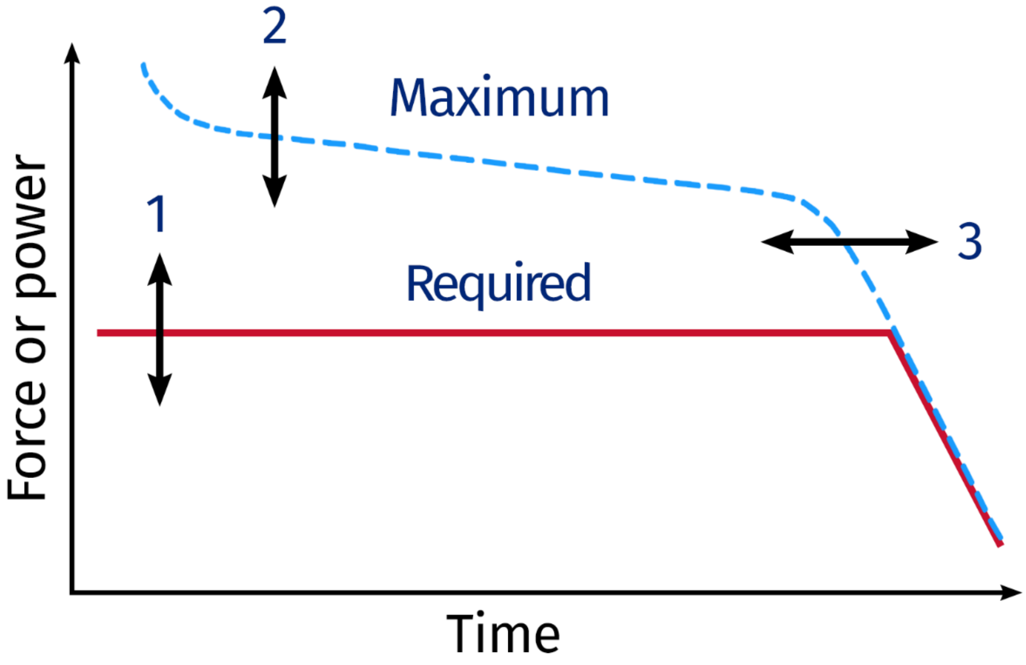

Figure 1.1.2.1 | Schematic to illustrate different mechanisms leading to exhaustion. Dashed line shows how the maximum force (or power) declines during repeated tetani. Solid red line indicates a submaximal force required for a particular activity. Exhaustion (failure to produce the required force) occurs at the intersection of the two lines (red and blue lines). Increases and decreases in the required force (arrow 1) will cause earlier and later onset of exhaustion, respectively. Increases and decreases in the maximum force that the muscle can produce (arrow 2) will also change the time to exhaustion. Finally, changes in the intrinsic fatiguability of the muscle (arrow 3) will also change the time to exhaustion. Repainted from Allen et al. 2008 (1).

At this point, it is necessary noticing that voluntary contractions are activated by complex pathways starting in the cortex, leading to the spinal cord, reaching the muscle's neuromuscular junction, and ending with muscle contractions. The processes happening inside the spinal cord and above are defined as central, whereas the processes occurring in the peripheral nerve, neuromuscular junction, and muscle are defined as peripheral. Fatigue can potentially arise at many points in this pathway, so that it can be helpful divide it into central and peripheral fatigue.

Peripheral fatigue

Researchers investigated ***peripheral fatigue*** by studying the principal changings occurring in the chain of events involved in excitation-contraction coupling in skeletal muscle. They supposed that a failure anywhere in this chain could contribute to developing peripheral fatigue.

ATP depletion was the first substance studied because it is considered the most directly involved in the transduction of chemical-free energy into mechanical work (28). Many subsequent kinds of research have reported that cytoplasmic [ATP] does not drop below 60% of the resting level during either imposed stimulation or voluntary exercise (3, 61, 72), though these values remain challenging to interpret (for a rev see Allen 2008 (1)).

Another substance of interest is inorganic phosphate (Pi) a product of ATP consumed during contraction. According to present models of cross-bridge force production, the myosin head is first bound weakly and firmly to the actin filament. After that, Pi is released, possibly resulting in a further increase in force production. This implies that increased Pi inhibits the transition to high force cross-bridge states, and fewer cross-bridges would be in high-force states when Pi increases during fatiguing stimulation. In line with this, experiments on skinned fibres consistently show a substantial decrease of force production in the presence of elevated Pi (45, 49).

Lactate is another historically suggested molecule causing muscle fatigue. Lactate is the primary source of acid production in skeletal muscle and a relationship between lactic acid production and muscle activity has long been recognised (20). This belief sinks its roots in a series of old studies (for a rev. see Fitts 1994 (20)), which related the production of lactic acid to contractile activity and leading the foundation for the hypothesis that lactic acid caused muscle fatigue. These previous works established that resting muscles in oxygen produced little lactate, while lactate production was high under anaerobic conditions. These authors suggested that the lactic acid may self-limit by inducing fatigue in the stimulated muscle. This concept was supported by connections between development and recovery from fatigue and the appearance and disappearance of lactic acid. Contrary, experiments with skinned muscle fibres have shown that lactate, at concentration even up to 50 mM, has relatively little effect on force production by the contractile apparatus (2, 17). Furthermore, it was shown that twitch and tetanic forces are virtually unchanged in the presence of 30 mM cytoplasmic lactate (Figure 1.1.2.2) (51).

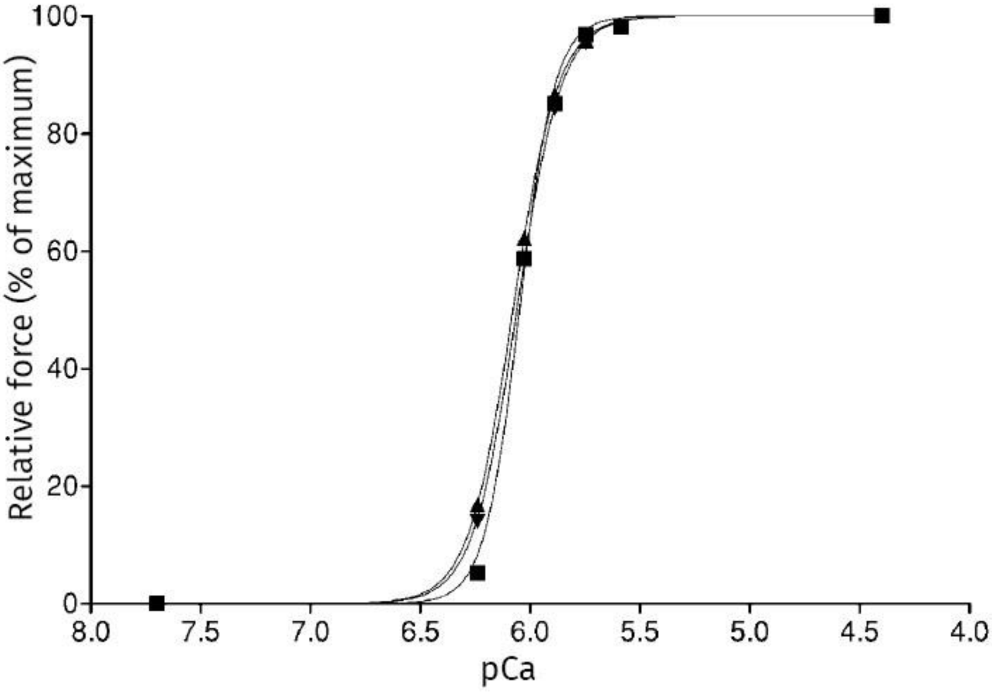

Figure 1.1.2.2 | Effect of 30 mM L(+)-lactate on the Ca2+ sensitivity of a single, mechanically skinned rat skeletal muscle fibre. The force/pCa relationship is shown before (▲), in the presence of 30 mM lactate (■) and after (▼) washout of lactate. The fitted curves were generated using the Hill equation. From Posterino et al. 2001 (51).

A large and rapidly growing literature establishes that reactive oxygen species (ROS) are produced in active muscles and have some role in fatigue. The most convincing evidence that ROS contributes to fatigue comes from experiments with exogenously added ROS scavengers, which reduce fatigue in isolated muscles of intact animals and humans (1).

The role of ROS in development of muscle fatigue was studied using N-acetylcysteine (NAC), a ROS scavenger, injected into the circulation.

In animals, NAC injection into the circulation does not affect the control force but increased the force at the end of a fatiguing electrical stimulation (20-Hz phrenic nerve stimulation) (59).

Highly similar results were also obtained in human subjects by Reid et al. (53). He observed that a pre-treatment with NAC intravenous infusion did not alter the function of unfatigued muscle: MVC performance and the muscles' force-frequency relationship was unchanged. Otherwise, during fatiguing contractions, NAC increased force output, an effect that was evident after 3 min of repetitive contraction and persisted throughout the 30-min protocol.

Contrasting results were obtained more recently by Medved and colleagues (43) who conducted a double-blind, crossover study investigating NAC's role in the time to fatigue during prolonged submaximal cycling exercise. The authors hypothesised a performance enhancement after NAC intravenous infusion, based on Reid's findings described above. Although they found no effect on time to fatigue in the whole group of subjects, a result probably remarked by different responsiveness of individuals to NAC. For the first time, this study showed that the effects of NAC on prolonged exercise are dependent on the VO2 peak, with a tendency for time to fatigue to be increased in the fitter subjects.

Central fatigue

We must not forget that just as many sites within the muscle cell control force, so many sites within the central nervous system (CNS) can modify the output of motoneurons.
Mosso, in the early 1900s, reported the first evidence of this purpose. He compared fatigue in voluntary and electrically induced contractions and measured central fatigue using various new techniques and devices such as Mosso's ergograph and Mosso's balance (Figure 1.1.2.3) (rediscovered by Sandrone et al. 2014 (56)). Over the years, the development of new neurophysiological techniques has allowed in-depth studies on central fatigue.

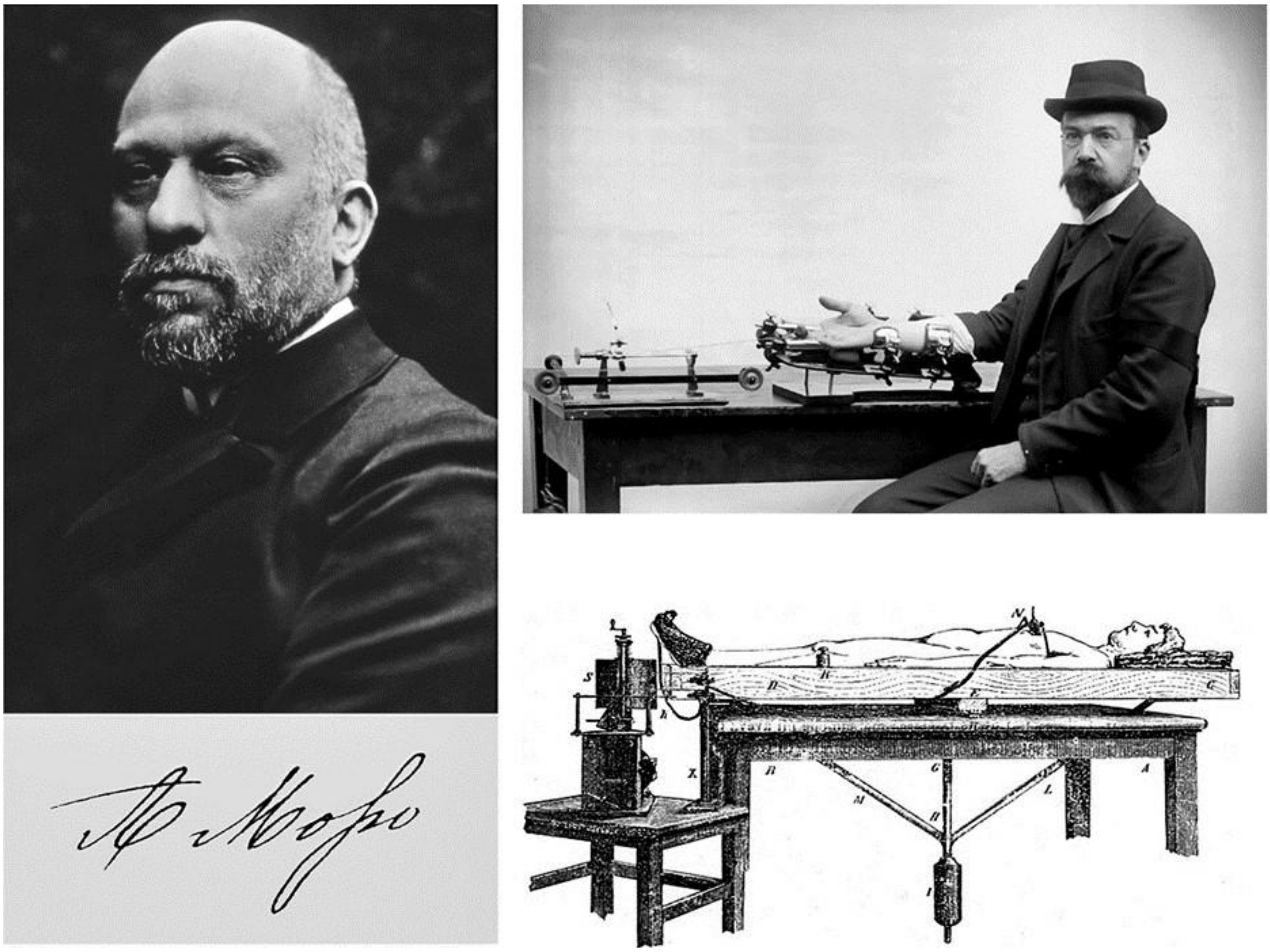

Figure 1.1.2.3 | On the left: photograph of Angelo Mosso and his signature (courtesy of Marco R. Galloni). On upper right: Angelo Mosso performing one of his experiments (courtesy of Marco R. Galloni). On bottom right: Mosso's 'human circulation balance', used to measure cerebral activity during resting and cognitive states (picture and figure Angelo Mosso's original drawing, modified and adapted from Mosso, 1884, Atti della Reale Accademia dei Lincei). From Sandrone et al. 2014 (56).

Studying central fatigue trough peripheral nerve stimulation

About 20 years later Reid (52) compared a maximal voluntary contraction (MVC) of finger flexion with force produced by electrical stimulation of the median nerve under isometric conditions. He found no marked difference from both forces disproving the standard view that the maximal contractile force was so high that "strength is kept in bounds by the inability of the higher centres to activate the muscles to the full" (44).

Merton (44) provided additional insight when he showed that the force increment added by a stimulus to the ulnar nerve was inversely proportional to the level of initial force during a voluntary effort. No force increment appeared when voluntary force approached maximal values.

Similarly, Newham et al. (47) observed an inverse but near linear relationship between the additional force elicited by the superimposed stimulation of the quadriceps muscle and the force contractions level ranging between 5 and 100% of subjects' maximal effort.

This proportional decline with increasing central drive, confirmed in later studies (for a rev. see Gandevia 2001 (21)), became a valid method to assess voluntary activation of muscle (Figure 1.1.2.4).

Studies used this so-called ***twitch interpolation*** technique, following a series of different methodological approaches (single, double, or train pulse superimposed stimulations).

Kent-Braun (30) found that a superimposed train of stimuli is more sensitive than either a superimposed single or double stimulus in detecting central activation failure during maximal voluntary isometric contractions.

Using this technique, it has been deduced that central fatigue contributed to the force loss experienced during sustained or repeated MVC (8, 30, 58).

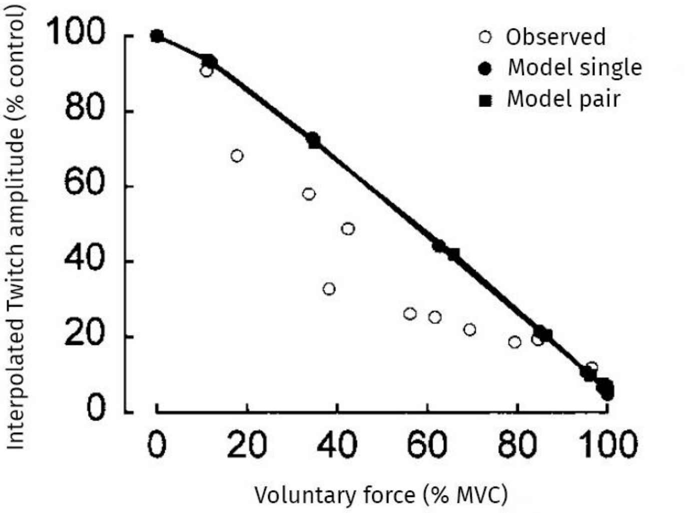

Figure 1.1.2.4 | Real and simulated twitch interpolation for adductor pollicis. amplitude of interpolated twitches (expressed as a percentage of resting twitch amplitude) at contraction intensities of 0–100% MVC. Experimental data are shown, as well as simulations with single and paired interpolated stimuli. From Gandevia 2001 (21).

Few authors have first studied this change of central activation during sustained contraction (5, 22). The oldest study conducted by Bigland-Ritchie et al. (6, 7) did not find any central activation failure in adductor pollicis. The second showed that central activation was kept optimal at the beginning of a 5-min sustained MVC of tibialis anterior and first dorsal interosseus muscles, but substantial failure occurred during the last 3 min. Gandevia's group was the only one that showed the development of central activation failure during a sustained maximal contraction in biceps brachii (22). They presented the superimposed force responses as a percentage of the voluntary force. This method did provide good insight into the development of central fatigue but did not allow quantification of force loss.

Recently, the interpolated twitch technique to assess central fatigue was slightly criticised (50, 58). This criticism is based on essential feature related to unit of measure. Since the level of voluntary activation is calculated through force's measure electrically evocated, use a superimposed force response to estimate the amount of failure of central drive does not consider the potential contribution of peripheral fatigue, which decreases the maximal possible force response (50, 58).

Schillings and colleagues (58) conducted a study to find the relative contributions of central and peripheral factors to fatigue during a maximal sustained 2-min voluntary contraction in healthy subjects. They used electrical stimulation and measurements of muscle fibre conduction velocity simultaneously. They discovered that peripheral and central fatigue does not change in parallel: during the first minute, the decline of voluntary force can be explained almost exclusively by peripheral factors. After about 1 min, however, peripheral fatigue levels off. Then, the further decrease of voluntary force can almost totally be attributed to central fatigue.

In addition, they tested two new methods to estimate central fatigue compensating the influence of peripheral fatigue: they normalised the superimposed force response by comparing it with the actual voluntary force and or by comparing it with the estimated linearly interpolated stimulated force. This last provides the most reliable quantification of central fatigue.

The decrease in voluntary activation observed in the studies described above could result from spinal or supraspinal mechanisms that alter the final output of force generated.

Researchers investigated muscle spindle, Golgi tendon organ, small-diameter muscle, presynaptic modulation motoneuron properties, and others at the spinal level.

Motoneurons possess an intrinsic property to adapt their discharge frequency to injected current (57). The discharge rate typically exhibited a rapid initial decline, characterised by a linear frequency-time relation, followed by a gradual exponential decline that continued for the duration of current injection. This behaviour is similar to the discharge frequency recorded in a single human motor unit during an MVC (Figure 1.1.2.5) (41).

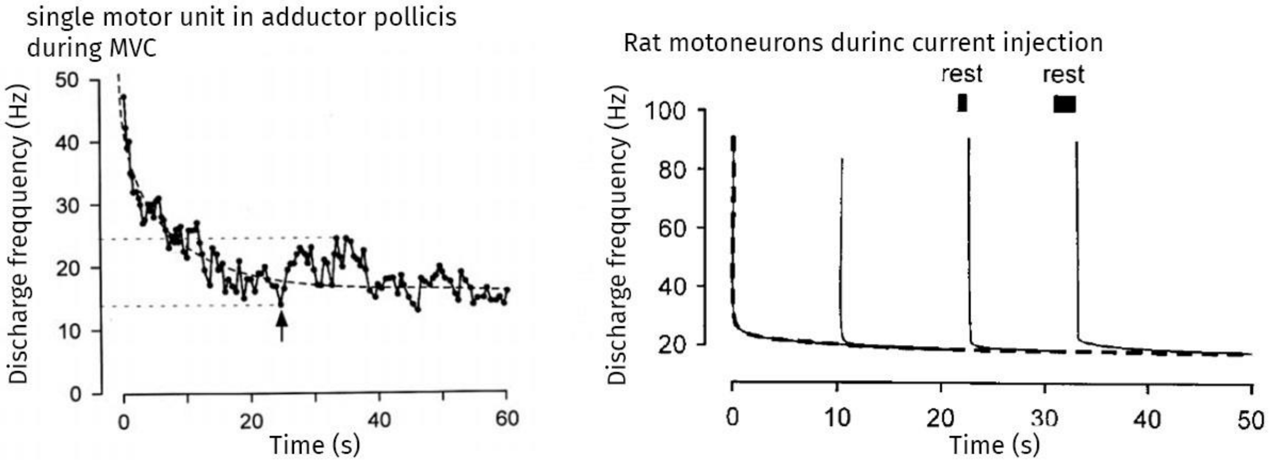

Figure 1.1.2.5 | On the left: discharge frequency of the same unit during the MVC is shown as the reciprocal of the period occupied by 10 consecutive spikes. Points were plotted at the end of the 10-spike period. A curve has been fitted to the decline in rate. The highest initial peak firing rate was 150 Hz. Arrow and horizontal dashed lines mark a period when discharge rate almost doubled, evidence of prior submaximal voluntary drive. On the right: dashed curve shows response to current injection lasting 1 min in rat motoneuron studied in vitro. If the stimulus current was briefly interrupted (“rest,” horizontal bars), the initial adaptation recovered. From Gandevia 2001 (21).

Other arguments suggested that muscle spindle inputs can facilitate human motoneurons during strong isometric contractions and fatigue (21). Two findings support this assumption. First, the firing rate of motor units decreases and becomes more irregular during a partial block of the motor nerve with a local anaesthetic that tends to block preferentially small fibres (25) (including fusimotor axons). Second, tendon vibration that activates muscle spindle endings can increase force during an isometric MVC of ankle dorsiflexors (Bongiovanni et al.1990). Unfortunately, these results should not necessarily be used to argue the decline in maximal motor unit firing rates during sustained MVCs due to reduced muscle spindle facilitation. Instead, it shows that augmentation of muscle spindle discharge facilitates motoneuron discharge when voluntary activation has declined during fatigue (21).

Group III and IV muscle afferents innervate free nerve endings plentiful and are distributed widely throughout the muscle. These afferents are either silent or maintain low background discharge rates. They respond to local mechanical, biochemical, and thermal

events. Many factors cause their discharge to increase during strong contractions and fatigue, mainly if the contraction intensity is sufficient to impair muscle perfusion.
Sinoway et al. (60) examined the interaction of lactic acid and static contraction on the discharge of group III muscle afferents. This work highlighted the critical role that lactic acid plays in group III muscle afferents during contractions. He showed that increased delivery of lactic acid in the absence of contraction enhances their discharge rate (impulse per minute). The reduction in lactic acid levels during contraction decrease their discharge. In addition, repeated exposure of the afferent to lactic acid leads opposite effects (reduction in discharge frequency).

Studying central fatigue trough transcranial magnetic stimulation

Supraspinal mechanism involved during muscle fatigue could be investigated through a wide range of tools available to study the corticomotor system. All neuroimaging techniques accommodate a series of advantages and disadvantages: positron emission tomography and functional magnetic resonance imaging, for example, provide a complete overview of the brain system but do not have an adequate temporal resolution to reveal the detailed time course of cortical activation. Therefore, during the investigation of specific cortical area (such as the motor cortex), more detailed information could be obtained using electroencephalography (EEG), magnetoencephalography (MEG) or transcranial magnetic stimulation (TMS), which have an excellent temporal resolution.
Transcranial magnetic stimulation is a non-invasive tool for the electrical stimulation of neural tissue, including the cerebral cortex, spinal roots, and cranial and peripheral nerves (31). It is used as a research tool to study many aspects of human brain physiology, including motor function, vision, language, and brain disorders' pathophysiology (24). When TMS is applied to the primary motor cortex, it produces descending volleys in the spinal cord. This, in turn, activates the spinal motoneurons, and a motor-evoked potential (MEP) can be recorded in the target muscle with surface electromyography (EMG) (Figure 1.1.2.6).

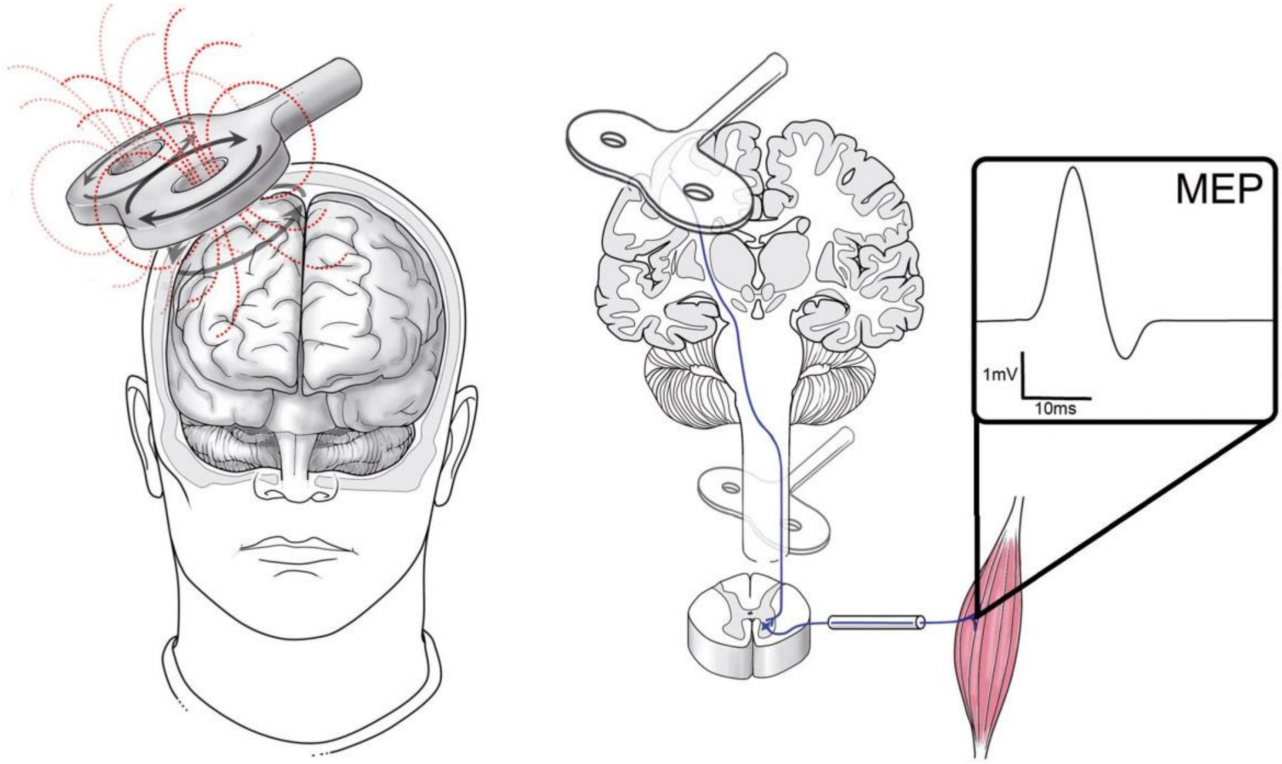

Figure 1.1.2.6 | On the left: transcutaneous magnetic stimulation (TMS). On the right: TMS stimulating a portion of the motor cortex, which travels down the spine and activates a muscle.

The size of the MEP in a muscle will depend on several factors, such as the "strength" of the corticofugal connections with motoneurons, the "excitability" of the underlying motor cortex and motoneurons, and the properties of the muscle fibre action potential.

Taylor and colleagues (64) investigated the excitability of the motor cortex during fatiguing contractions of the elbow flexors in human subjects. He noted that during sustained contractions at 30 and 100% MVC, the short-latency EMG evoked in biceps brachii, and brachioradialis by TMS increased in size. He also observed that when the blood supply to the brachioradialis was blocked with a sphygmomanometer cuff at the end of a sustained MVC, the changes in EMG responses to transcranial stimulation rapidly returned to control values. This finding suggests that changes in these responses during fatigue were not mediated by small-diameter muscle afferents disproving the precedent results on group III muscle afferents obtained by Sinoway (60). Latella (33) recently confirmed this hypothesis investigating the effect of group III muscle afferent feedback on the excitability of intracortical neuronal networks in human muscle. His work's results do not support the idea that actions of group III/IV afferents on cortical motor networks contribute to the reduction in voluntary activation.

A few years later the study of Taylor, the same group (65) tested whether changes in response to stimulation of the peripheral motor nerve could fully account for the increase in the MEP size previously observed during fatigue. During MVCs were recorded the MEP and the response to supramaximal stimulation of motor nerve fibres in the brachial plexus. M-waves evoked by supramaximal nerve stimuli grew during a sustained voluntary contraction. However, the M-wave's growth was less than that of the MEP and did not fully account for the increase in the MEP size. This result confirms that changes within the central nervous system contribute to the increased excitatory response to cortical motor stimulation during fatigue. It was also observer an increase in onset of MEP's latency.

The increase in MEP size during fatigue were explained as changes in the central nervous system, while the increased latency of the MEPs and lengthening of the peripherally evoked silent period was described as a decreased excitability of the alpha motoneuron pool.

This prolongation in silent period, also observed in other studies, was later reinterpreted by Gandevia (21). He assumed that prolongation in the silent period reflects a balance at the corticofugal cell between local intracortical inhibition and excitation evoked by the stimulus and excitation derived from volition (71). This phenomenon could be caused by a net increase in intracortical inhibition associated with the stimulus and a reduced voluntary drive to the cortical output cell. The prolongation is not due to inadequate volitional drive to the motor cortex because the silent period prolongation recovers more quickly than central fatigue and can also be dissociated from central fatigue during intermittent contractions (22).

Concerning the MEP size, several works observed an increase during near-maximal efforts and a return to control levels during recovery of brief MVCs. Contrary, MEPs becomes depressed for up to half an hour when evoked in relaxed muscles. These consistent changes in the MEP size could be due to the ability of TMS to evoke different numbers of I-waves (indirect volleys occurring at intervals of about 1.5 ms from transcranial stimulations), which modify the motoneuron pool output (providing an inhibition effect at supraspinal levels).

Stimulation of the descending motor tracts offers a simplification because single stimuli can be given to a population of rapidly conducting axons far from the cell body with the evoked response. This technique consists of magnetic stimulation through a double-cone coil centred close to the inion. Descending tract axons activated excite motor roots innervating arm muscles, producing a motor response (CMEP) similar to those recorded by the cortex stimulation (MEP).

Weavil (73) used this technique to investigate the influence of fatigue on corticospinal excitability during cycling exercise. He analysed MEP, CMEP, and M waves obtained by subjects' quadriceps during brief non-fatiguing cycling bouts or cycling to exhaustion. During non-fatiguing cycling, the area of MEPs and CMEPs, normalised to M waves, similarly increased in the quadriceps (Figure 1.1.2.7). In contrast, there was no change in normalised MEPs and CMEPs during fatiguing cycling.

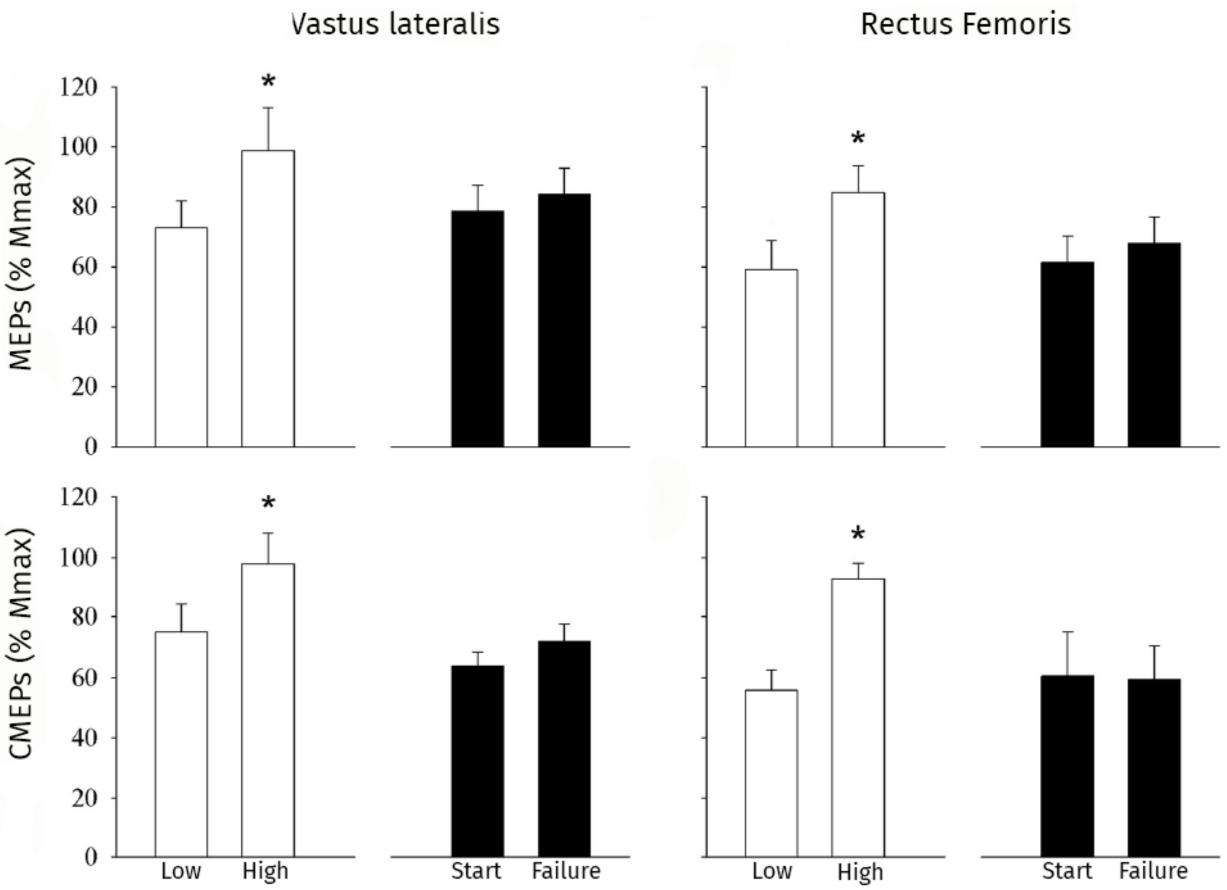

Figure 1.1.2.7 | Changes in the excitability of the motor pathway (area of MEP normalized to Mmax), the motoneuron pool (CMEP normalized to Mmax) normalized by the maximal M waves obtained by subjects' quadriceps femoris in different conditions. From Weavil et al. 2016 (73)

He concluded that the unaltered excitability of the corticospinal pathway from the start of intense cycling exercise to exhaustion is, in part, determined by inhibitory influences on spinal motoneurons obscuring the facilitating effects of muscle activation.

More recently Vernillo (70) showed that motoneuron recovery behaviour depends on the muscle performing the exercise by comparing the changes in motoneuron excitability in elbow-flexor and knee-extensor muscles after sustained fatiguing maximal voluntary contractions. A different recovery pattern in motoneuron excitability occurs in elbow flexors as it recovered by 60 s, whereas knee extensors were unaffected by fatigue, as precedent observed by Weavil (73).

Studying central fatigue trough functional magnetic resonance imaging and electroencephalography

Despite muscle fatigue being mostly investigated in the corticomotor system, recent research shifted its focus to different brain areas. A significant scientific contribution came from Liu, who investigated muscle fatigue through functional magnetic resonance imaging (fMRI) and electroencephalography (EEG).

In 2002 he studied the brain activation by fMRI during a sustained (2-min) maximal-effort handgrip contraction while handgrip force and finger muscle electromyographic (EMG) data were recorded simultaneously. Handgrip force and EMG signals declined in parallel during muscle fatigue, but fMRI-measured brain activities first substantially increased and then decreased. This similar signal modulation occurred in the primary sensorimotor areas and the secondary and association cortices (supplementary motor, prefrontal, and cingulate areas), suggesting integrated information processing in many cortical regions.

One year later (37), he quantified brain activation during submaximal fatigue muscle contractions using the same devices described above. Subjects performed a sustained handgrip contraction and intermittent handgrip contractions. For the sustained contraction, EMG signals increased linearly while the target force was maintained. The cortical activities in the contralateral sensorimotor cortex increased sharply firstly, then plateaued during the last phase. For the intermittent contractions, the EMG signals increased during first and then began to decline, while the handgrip force also showed a sign of decrease despite maximal effort to maintain the force. As in sustained contraction, the fMRI signal showed a linear rise for most of the task and a plateau at the end. These similarities in fMRI signals obtained in both tasks showed that the brain increased its output to reinforce the muscle to continue the performance and possibly process additional sensory information.

Liu obtained different results two years later when tested intermittent MVCs (38). In this study level of the fMRI signal in the primary (sensorimotor), secondary (supplementary motor), and association (prefrontal and cingulate) motor-function cortices did not change significantly throughout the fatigue task (although the signal of the primary sensorimotor cortex showed a clear trend of decline).

These findings, taken together with previous work results, support the notion that the cortical motor centres control the tasks of repetitive and continuous muscle contractions differently. There is a decoupling in the signal changes of the brain and during muscle fatigue processes induced by maximal voluntary contractions.

In 2007 he conducted his first EEG study (36). In this research, six healthy men participated in two experiments. First, they performed 200 intermittent handgrip MVCs of the right arm. In the second, they also executed 200 handgrips MVCs but with a much more extended rest period (30s instead of 5s). He estimated the changes in the brain's source locations, collecting high-density (64 channels) scalp EEG signals during both experiments. The centre of brain activation showed substantial location shifts during the fatigue motor task. Compared to the non-fatigue condition, the weighted centre of the source locations for all the participants shifted toward the right hemisphere (ipsilateral to the muscle activation), anterior, and inferior cortical regions under the fatigue condition.

Taghizadeh et al. (62) investigated the effects of muscle contraction and fatigue induced by two different percentages of MVC on EEG signals. EEG signals were recorded from twenty-one healthy human subjects during three phases (rest, pre-fatigue, and post fatigue) contraction of adductor pollicis muscle at 30% and 70% MVC. The authors obtained no significant change between three states in all EEG bands (alpha, beta, and gamma).

Mental fatigue

Mental fatigue refers to the feeling that people may experience after or during prolonged periods of cognitive activity. These feelings generally involve tiredness or even exhaustion, an aversion to continuing with the current activity, and a decrease in the level of commitment to the task at hand (54).

Unfortunately, mental fatigue appears to be a highly complex phenomenon that involves changes in mood, information processing and behaviour, making it as complex subject to study (11).

For this reason, mental fatigue was very differently investigated through the years, for example using the rate of perceived exertion scale, questionaries, telephone surveys, and cognitive tests.

Thorndike was the first scientist who studied the effects of mental fatigue researching a decline in cognitive performance (67). He tested the pupils before and after a school-session with four tests assigned in a different order: a set of multiplication examples to be done in a given time, a page of printed matter full of misspelt words which were to be marked in a given time, two sets of nonsense syllables to be written from memory after ten seconds' look at them, and two sets of figures and one set of simple forms (e. g., square, triangle) to be written from memory in the same way. After having not found significant differences in cognitive performance, he concluded that the mental work of the school day does not produce mental fatigue.

More promising results were obtained when mental fatigue was induced through sustained cognitive tasks called ***time on task*** (TOT).

In 1986 Nordqvist (48) applied this method requesting to subjects to read texts for a period of two hours. The reading of texts was presented by means of a videotex system or by means of print on paper. He observed a slightly decrease in the number of pages read for both groups with the task’s progressing. Also, free recall performance decrease when task became more demanding. However, other reading performances do not change trough the experiment. These results demonstrated only marginal effects of fatigue.

Lorist (40) examined the effects of mental fatigue on planning and preparation for future actions using a task-switching paradigm. Fatigue was induced by TOT, with subjects performing a switch task continuously for two hours. The switch task seemed to be demanding in terms of effort investment and attentional requirements, as reflected in increased fatigue levels and decreased vigour levels (investigated with mood questionaries) with TOT. With increasing mental fatigue, preparation processes seemed insufficient, and the number of errors increased. Also, reaction times became longer with increasing mental fatigue (Figure 1.1.2.8).

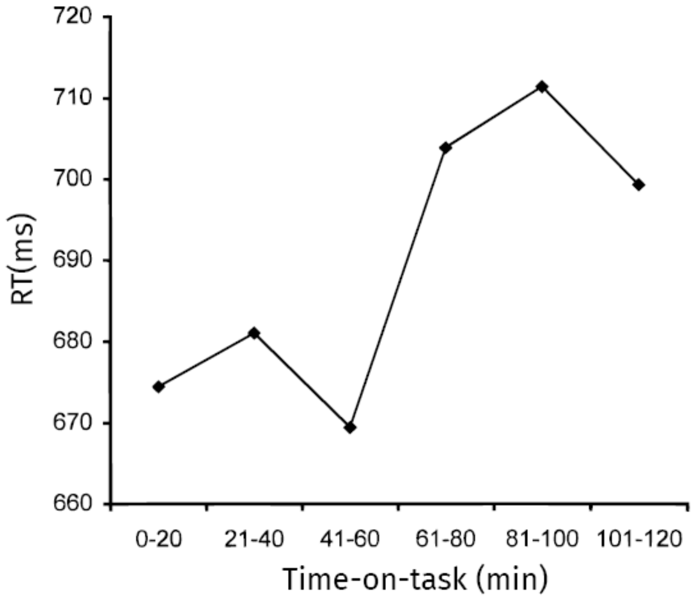

Figure 1.1.2.8 | Mean reaction times (RTs) depicted as a function of time on task. The RTs are averaged across different response–stimulus interval (RSI) conditions and trial types. From Lorist et al. 2000 (40).

Lim and colleagues (34) showed that in a group of 15 subjects who performed a 20-min continuous psychomotor vigilance test, subjects displayed progressively slower reaction times and a significant increase in mental fatigue ratings after the task.

Holtzer et al. (26) examined the effect of mental fatigue on the Attention Networks Test in a group of 228 non-demented older adults. To address the decline in cognitive performance of ageing population, authors used a variant of the original Attentional Networks Test (19) that enhanced the size of the stimuli. A significant cognitive fatigue effect was observed only in executive attention. This finding provides empirical support to a theoretical model implicating the frontal basal ganglia circuitry as a core substrate of mental fatigue.

These increasing in reaction time induced by mental fatigue, were not reflected in the temporal preparation anticipating participant's response. Indeed, when Langner et al. (32) presented to subjects a variable foreperiod paradigm (a task in which a warning signal was presented before an imperative stimulus marking the beginning the response), he observed that TOT-induced mental fatigue did not influence the pattern of response timing. This suggest that TOT-induced mental fatigue generally reduces cognitive efficiency but unaffected temporal preparation's leaves under time uncertainty.

Today, declines in executive functions such as executive attention (26, 35), sustained attention (32, 34), goal-directed attention (9), alternating attention (69), divided attention (68), response inhibition (29), planning (39, 40), and novelty processing (42) are considered a common feature of mental fatigue.

Among the various components of executive function, selective attention, particularly conflict-controlling selective attention (response inhibition), is highly vulnerable to mental fatigue (63). Conflict-controlling selective attention was evaluated using reaction time and error rate of Stroop-like trials in a traffic-light task and was impaired after performance of a fatigue-inducing mental task, even though cognitive task performance related to executive functions such as alternating attention, divided attention, sustained attention, and working memory was not altered (63).

In 2005 Lorist (9) suggested that this impaired cognitive control could results from a failure to maintain adequate levels of dopaminergic transmission to the striatum and the anterior cingulate cortex (ACC). Indeed, he documented that mental fatigue results in compromised error monitoring reflecting in a significant attenuation in EEG measurements (ERN) and adjustment failures in post-error performance. During two hours of prolonged cognitive task, subjects seemed to compensate the reduction in performance efficiency by increasing RTs, but preventing the occurrence of errors and related demands placed on ACC.

The capacity to monitor error performance is thought to rely predominantly on intact ACC functioning (14, 23). In addition to the monitor function, the ACC serves to signal the need for enhanced cognitive control after a performance error has occurred. The present data provided initial support for the notion that mental fatigue involves dopamine-mediated mechanisms of cognitive control, thereby providing the initial contours of a neurocognitive theory of mental fatigue.

Differently from muscle fatigue, which results more steady, mental fatigue could be modified from external inputs such as a reward which enhance subjective motivation.

Chaudhuri and Behan (15) noted that in their patients' fatigue is, at least in part, due to a deranged motivation in self-initiated tasks. Boksem and Tops (11) proposed that mental fatigue can be viewed as an effort/reward imbalance: as long as one feels that the invested effort in the end will result in sufficient rewards, one will continue working. However, when the perceived effort becomes too great and the reward no longer compares to this, the motivation to continue will dissipate and one will want to disengage from the task, feeling fatigued.

The approach of potential rewards and the avoidance of potential punishment are fundamental to all goal directed behaviour (46): for something to constitute a goal, it must be associated with a high value of predicted reward and low potential risk.

Boksem et al. (10) tested this hypothesis manipulating subjects' motivation by offering them a certain amount of money if they performed well in the remainder of the task. As previous studies authors monitored subjects' neurophysiological and behavioural indices after a prolonged demanding cognitive task (lasting two hours). In this experiment, fatigued subjects did not all respond to the motivational instructions in the same manner. When the two hours of cognitive task ended, the instructions were to stress both speed and accuracy, but subjects almost never improved both. opting instead to improving on one measure by sacrificing the other. In addition, their strategy to improve performance (speed vs accuracy) reflect an increase of specific neurophysiological indices. It appears that fatigued subjects, when motivated, could once again monitor their actions adequately (accuracy). However, in doing so, they had to sacrifice their response speed. On the other hand, fatigued subjects that were, when motivated, once again able to increase their response speed, appeared unable to monitor their actions, resulting in increasing numbers of errors.

A similar study conducted few years later (39) demonstrated comparable effects of mental fatigue on performance and brain activity. In addition, in this study, Lorist proposed a possible modulation of the dopamine system to explain the observed the fatigue and motivation effects.

The dopamine activity appears to depend on the predictability of the reward, such that unpredicted rewards elicit dopamine activation (positive prediction error) and an unpredicted nonreward induces a depression in dopamine activity (negative prediction error), while fully predicted rewards do not elicit dopamine activity (for a rev see Boksem et al. 2008(11)). Although dopamine activity has mostly been associated with reward processing, it has been shown that mesolimbic dopamine neurons not only carry a reward signal but respond to a large category of salient and arousing events, including appetitive, aversive, high intensity, and novel stimuli (27), suggesting a more general role for dopamine in motivated behaviour (4, 16).Considering all findings presented in this paragraph, we can assume that muscle or mental fatigue impair the corresponding performance overusing it in intermittent or continuous task. The impairment is recovered after a rest period which bring back the performance to the initial level. Muscle fatigue is the most studied and documented probably because it is faster to elicit and more controllable than mental fatigue. Indeed, this last one seems to occur only after very prolonged period of sustained cognitive task (2 hours) and could be affected by changes in mood state.

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1.1.3 Motor imagery and action observation

Motor imagery (MI), defined as "the mental simulation of action without its actual execution" (32) and *action observation* (AO), defined as the process of observing actions performed by other, are two mental techniques able to activate the sensorymotor system. Researchers successfully applied they as motor learning methods (3, 5, 6, 21, 46) and literature still accumulates evidence of an AO- and MI-induced brain plasticity, especially in neurophysiological patients (2, 42).

Motor imagery

Since the 1930s, despite the term "motor imagery" had not yet been coined, research started to investigate the possible role of "mental practice" in motor skills development. In these previous studies, mental practice refers to a form of practice in which subjects produce a vivid mental image of performing a technique.

Jacobsen (30) pointed that a discharge in target muscles always follow the mental image of movement. Unfortunately, if we want to discriminate between movement execution and imagery, these discharges must be avoided. Consequently, researchers, in the following years, instructed subjects not to execute any movements of the target muscles and controlled this behaviour by electromyography (EMG) feedback.

During the 1960s and early 1970s, mental practice held a conspicuously prominent place in sport psychology and literature. The research design conducted in these years typically compared subjects' performances with previous mental practice with those who had not received mental instructions. A practice period of varying length was then instituted in which all groups, except the controls, practised (mentally or physically) a motor skill daily. Following this "training" period, the subjects' skills were tested under standard conditions to determine whether their performance scores differed as results of practice condition administered. Since the mental practice group surpassed the control group's performance, the mental practice was said to be effective in facilitating performance. Many studies have noted this finding (for a rev see Feltz et al. 1983), but it was sometimes found that mental practice groups did not perform as well as physical practise groups or the groups combining mental and physical practice.

In 1989 Decety (17, 18) observed that the duration of a mental simulation is similar to the execution also when participants load 25 kg on their shoulders. Results revealed, in all cases, that subjects took almost the same time to perform the real or the mental movement (Figure 1.1.3.1).

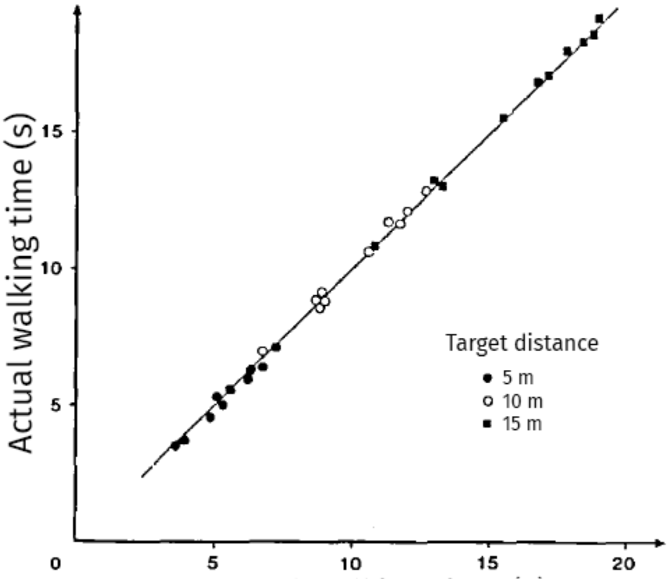


Figure 1.1.3.1 | Intrasubject distribution of mean mental walking times plotted against mean actual walking times. From Decety et al. 1989 (17).

This phenomenon next resulted related to central processing rather than the ability to execute a movement. Indeed, compared to healthy controls, patients with lesions of the motor cortex and patients with Parkinson's disease (22) showed decreased movement velocity when performed physically or mentally. Contrariwise, patients with spinal lesions only show a prolonged duration of movement execution but the same duration of imaged movement (15).

A few years later (16), he also provided evidence of autonomic response to motor imagery. He observed that cardiac and respiratory activity measurements during actual and mental locomotion at increasing speeds revealed a covariation of heart rate and pulmonary ventilation with the degree of imagined effort. The degree of vegetative activation of a subject mentally running at 12 km/h was comparable to that of a subject actually walking at 5 km/h (Figure 1.1.3.2).

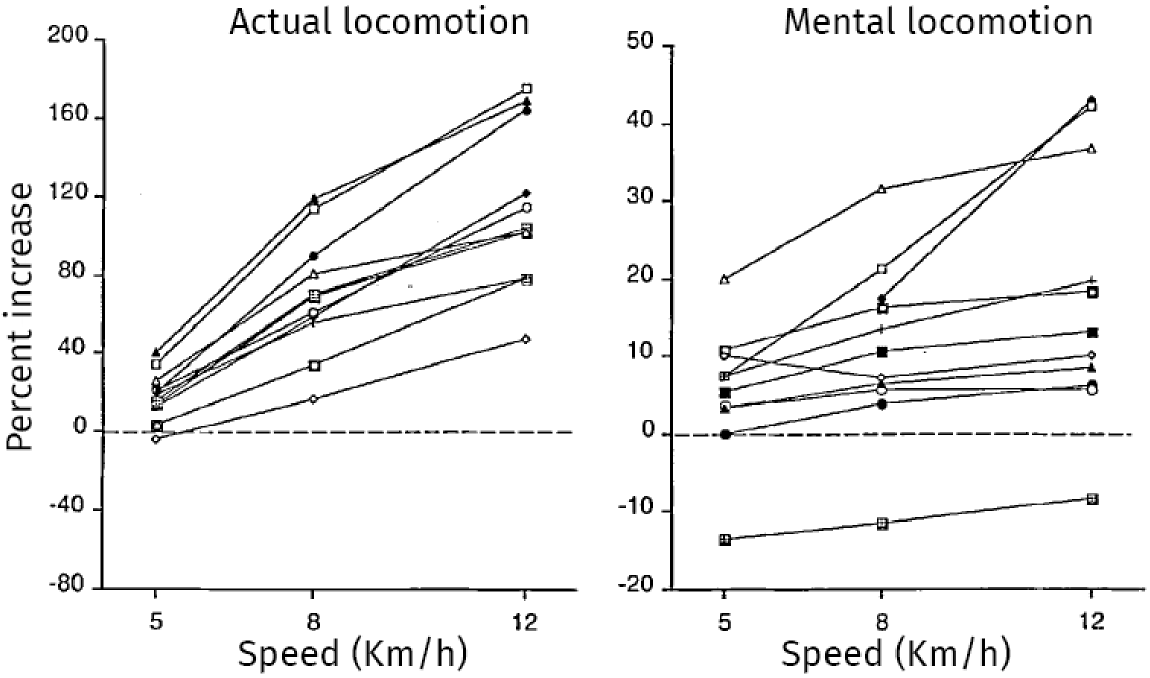


Figure 1.1.3.2 | Increase in heart rate during actual (left) and mental (right) effort, as a function of speed, in the 11 subjects. Values are expressed in percent of resting value. From Decety et al 1991 (16).

The autonomic response to imagined exercise was confirmed the year later by Wang and Morgan (53). Their results indicated that motor imagery produces a significant increase in ventilation and a significant elevation in systolic blood pressure.

Linking together his findings, Decety (14) proposed that the significant increase in autonomic response observed during imagined activities had a central origin, like a mind deception, leading the body to believe that some movements are being executed.

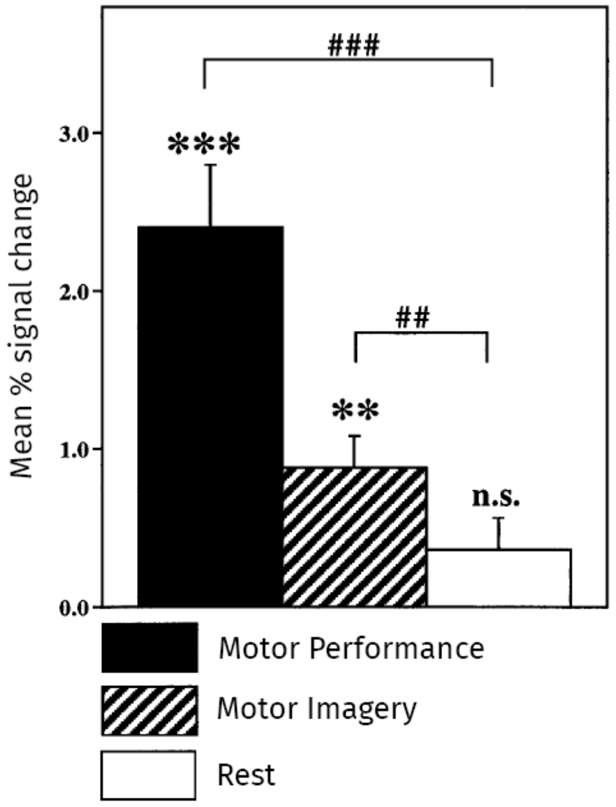
The evidence obtained in these years argued Jeannerod (37) to introduce distinct codes for mental activity (one for representing mental images and another for linguistic propositions), challenging previous assumptions that assess mental imagery as inseparable from general prepositional activity. One year later (31), he concluded that conscious motor imagery and unconscious motor preparation share common mechanisms and are functionally equivalent.

To date, motor imagery (MI; a term coined in 1994 by Jeannerod (37)) is considered a unique form of motor behaviour, intermediate in the continuum extending from motor preparation to movement execution (40).

This view is supported by increasing evidence showing a great overlap in brain regions' activation during MI and movement execution. Researchers observed this overlap in the primary motor cortex, pre-supplementary and supplementary motor areas, the premotor cortex, and in different parietal lobules' regions through the years (see Figure 1.1.3; paragraph 1.1; p 15).

Since the 1980s, fMRI and positive emission tomography (PET) have been the most used MI-related brain imaging research techniques.

Porro et al. (39), using an fMRI technique, found a significant increase in primary motor cortex activation following finger movements performed with actual and mental activity. A weaker activation was found during the motor imagery performance, which reaches about 30% of the activity observed in actual motor performance (Figure 1.1.3.3).



Highly similar results were contemporarily observed by Roth (45), who investigated the right and the left hand.

These two works were in contrast with the other two conducted in the same years, which employed PET to assess brain activity (19, 50). Stephan (50) suggested that the neural substrate of motor imagery differs from that involved in its execution by the absence of activation of the primary motor cortex.

These studies, which did not find significant activation in the primary motor cortex during MI, were later criticized for their adopted materials. The low temporal resolution of PET did not allow experimenters to catch the shorter cortical activation elicited by MI (35).

Figure 1.1.3.3 | Mean ± SEM percentage fMRI signal changes in the Pre-CG Post during actual or imagined motor performance or rest, relative to mean values of the control condition. Significant differences from values of the VI task at **p < 0.01 and *** p< 0.001, respectively; n.s., not significant. Significant differences at ## p < 0.01 and ### p < 0.001, respectively. From Porro et al. 1996 (39)

TMS represents a valid alternative to examine primary motor cortex activation. Its excellent temporal resolution permitted researchers to provide further evidence of the sensorimotor circuits' involvement during MI. Most of the studies in this field used the single-pulse TMS over the primary motor cortex during MI or before and after a mental training involving it. Modifications in corticospinal excitability are reflected in variations in the MEP amplitude. An increase in MEP size is interpreted as motor facilitation due to decreased cortical motor threshold or a greater number of recruited motor neurons (33).

Fadiga (23) spotted an increase in the MEP size during MI. Magnetic stimulation of the left motor cortex revealed increased corticospinal excitability when subjects imagined ipsilateral and contralateral hand movements. By contrast, the stimulation of the right motor cortex revealed a facilitatory effect induced by the imagery of contralateral hand movements only.

Rossini (44) explored corticospinal excitability of right abductor digit minimi (ADM) and first dorsal interosseous (FDI) muscles, sharing the same peripheral innervation but engaged in two different motor demands. Subjects' corticospinal excitability was tested under four different conditions: complete muscular and mental relaxation, mental simulation of the selective index finger or little finger abduction, mental non-motor activity (arithmetical calculation), and real motor task (slight and index finger abduction). The study showed that MI could produce specific facilitation on the prime-mover muscle implicated in the mentally simulated movement. This is mainly evident on FDI muscle, which controls fingers (i.e. the index) with highly corticalized motor representation.

This MEPs amplitude specific modulation for the muscle involved in the imagined action is a widely consolidated topic in recent literature (51).

In addition to this muscle-specific modulation, cortical excitability during MI manifests also changes according to different action phases and contexts. For example, Stinear and colleagues (52) remarked that kinesthetic (when a person focuses on the sensory information generated during actual action execution, including force and effort) and visual (self-visualization of a movement from a first- or third-person perspective) imagery strategies have differential effects upon corticospinal excitability. Only kinesthetic MI produced muscle-specific and temporally modulated facilitation of corticospinal excitability above resting levels, whereas visual MI of the same movement had no significant effects (Figure 1.1.3.4).

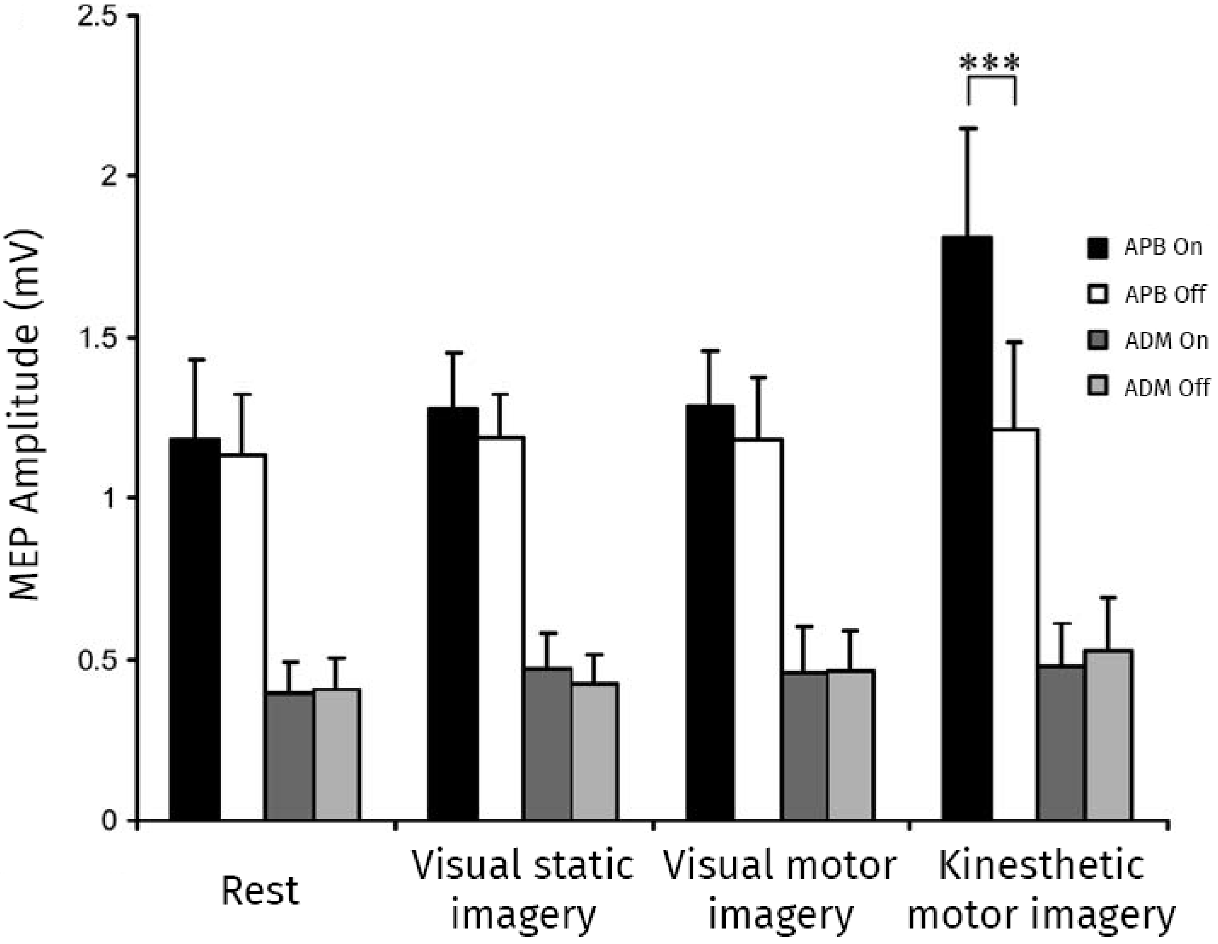


Figure 1.1.3.4 | Mean MEP amplitude of APB muscle as function of muscle involved in the imagined movement, phase, and task. TMS were delivered either 50 ms before the metronome beep (the ON phase), or 450 ms after the metronome beep (the OFF phase). The significant facilitation of APB MEP amplitude occurs only during the ON phase of kinesthetic motor imagery. From Stinear et al. 2006 (51).

This functional end neurophysiological equivalence between MI and motor execution led neuroscientists to evaluate its potential role in developing motor learning.

Gentili and colleagues (27–29), in a series of studies, tested the efficiency of mental and physical practice in improving motor performance. The improvement was evaluated by comparing the duration of overt (executed) and covert (imagined) movements during a motor learning paradigm. The task consisted of reaching and pointing movement in random order. In the first study (28), the authors observed a significant decrease in participants' movement duration induced by mental practice. However, according to previous studies, the motor performance improvement was lower after mental practice. In a second study (27), the authors evaluated the learning curve by comparing the trial-by-trial decrease duration of the executed and imagined movements. Although the learning rate was lower in mental practice than in physical practice, movement duration significantly decreased in both groups, and this gain lasted until 24 hours after. This finding was replicated in a third study (29), which marked greater improvements in the dominant arm's movement speed when compared with the non-dominant arm's performance. In addition to those reported between the 1960s and 1990s, these findings reinforced the idea that mental and physical practice elicits similar but not equal phenomena underlies motor learning.

Sanes (47) sustained that neuroplasticity (the brain's ability to change and adapt as a result of experience) is a necessary step to reach before obtaining motor learning. Also, the definition of structural plasticity (the brain's ability to actually change its physical structure as a result of learning) implies a strong correlation with it. Therefore, it could be premature to affirm that MI induced motor learning without reporting neurophysiological measurements indicating plasticity.

One of this first neurophysiological evidence was detailed by Pascual-Leone (38). They evaluated MEP amplitude modulation during the acquisition of new fine motor skills. Subjects were divided into three groups: a physical practice group, a mental practice group, and a control group. The physical and mental practice groups had to learn a piano sequence training it physically or mentally (according to the group assigned). The control group did not practice any sequence. Motor performance was monitored daily by testing the number of errors committed during participants' piano sequences execution.

Furthermore, authors acquired the cortical maps of the muscles involved in this activity to evaluate changes in primary motor cortex excitability. In the physical and mental practice groups, the number of errors decreased daily, and the cortical area dedicated to involved muscle significantly increased (Figure 1.1.3.5). These results were not observed in the control group, suggesting that mental practice, like physical practice, modulated the cortical motor representation of the trained muscles, evoking cortical plasticity.

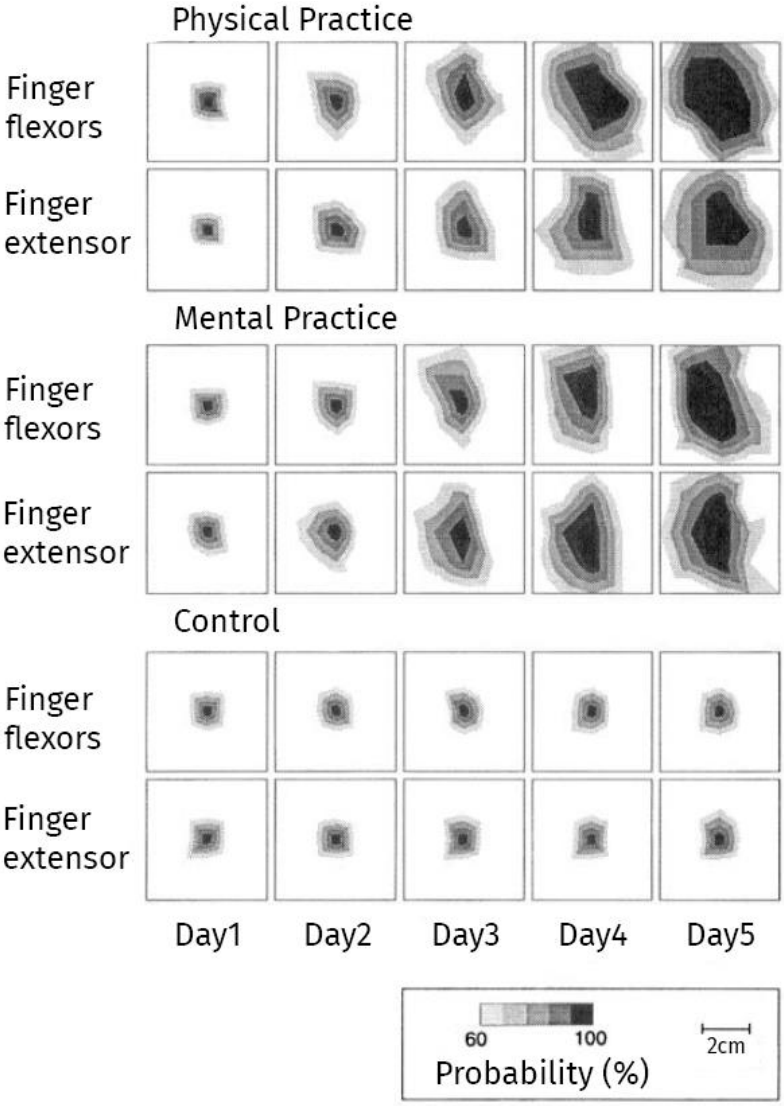


Figure 1.1.3.5 | representative examples of the cortical motor output maps for the long finger flexor and extensor muscles on days I-5 in a subject from each group. Each map is based on 25 measured points. From Pascual-Leone and Brasil-Neto 1995 (38).

Although this experimental evidence suggested that the neural mechanisms activated during MI and movement execution are similar, one crucial point differentiating imaged and actual movements is the lack of peripheral sensory feedback during MI. To provide this sensory feedback, Bonassi and colleagues (9) merged MI with peripheral electrical stimulation (ES) and tested MEP amplitudes and movement rate before and after this combined protocol (ESMI), MI alone and physical practice alone (PP). The task consisted of executing (PP) or imagining (MI and ESMI) finger-tapping movements at increasing rates. Results showed that similarly to PP, ESMI increased movement rate and MEP amplitude, whilst no differences were observed after MI alone, thus suggesting the crucial role of sensory afferents during mental practice to evoke plastic changes and motor learning

Action observation

Action observation (AO), defined as the process of observing actions performed by other, share several common properties with motor imagery. For this reason, research frequently studied both topics together. Nevertheless, AO showed the ability to activate motor areas slightly before MI. Clear evidence was reported firstly in the 1990s by a group of Italian neuroscientists working on macaque monkeys.

In 1992, Pellegrino et al. (20) published a case report study spotlighting a striking feature of many monkey's neurons. He identified special groups of neurons located in area F5 of the ventral premotor cortex which discharge not only when a monkey is performing an action, but also when the monkey passively observes a similar action performed by other. Pellegrini noticed a clear link between the effective observed movement and that executed by the monkey and, often, only movements of the experimenter identical to those controlled by a given neuron were able to activate it.

The same group of authors, few years later (25), deepened these results recording the electrical activity from 532 neurons of two macaque monkeys. After replicated the results previously obtained, authors named this set of F5 neurons “mirror neurons” (MN). This newly discovery presented a series of noteworthy features. For example, to be visually triggered, MN required an interaction between the agent of the action and the object of it, the sight of the agent alone or of the object alone (three-dimensional objects, food) were ineffective, and the actions most represented were grasping, manipulating, and placing (Figure 1.1.3.6).

Authors hypothesized the possible role of this system in action recognition and (given the proposed homology between F5 and human Brocca 's region) supposed the existence of similar matching system in humans.

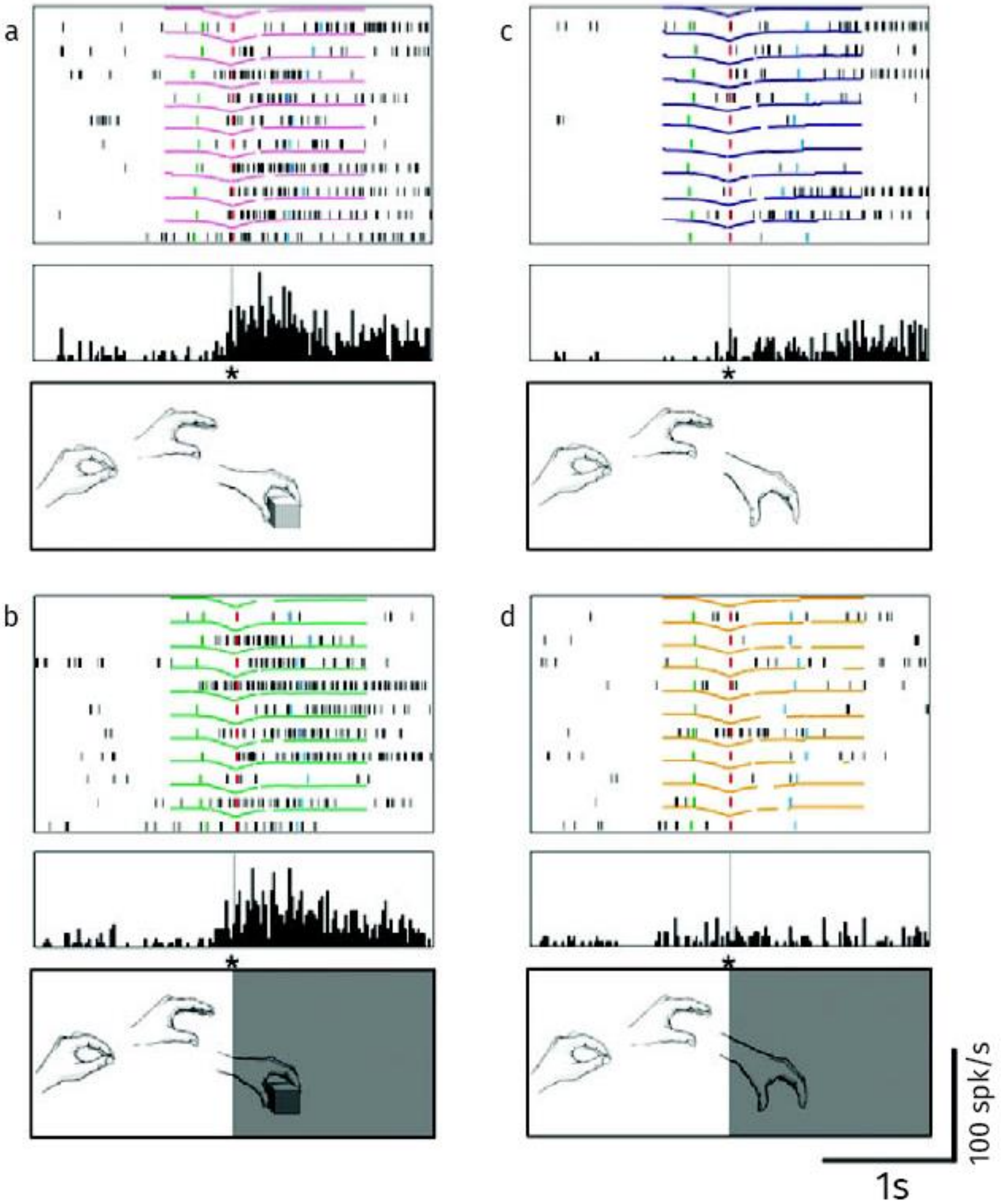


Figure 1.1.3.6 | Mirror neuron responses to action observation in full vision (a and c) and in hidden condition (b and d). The lower part of each panel illustrates schematically the experimenter's action as observed from the monkey's vantage point. The asterisk indicates the location of a stationary marker attached to the frame. In hidden conditions the experimenter's hand started to disappear from the monkey's vision when crossing this marker. In each panel above the illustration of the experimenter's hand, raster displays and histograms of ten consecutive trials recorded are shown. Above each raster, the colored line represents the kinematics of the experimenter's hand movements expressed as the distance between the hand of the experimenter and the stationary marker over time. Rasters and histograms are aligned with the moment when the experimenter's hand was closest to the marker. Green vertical line: movement onset; red vertical line: marker crossing; blue vertical line: contact with the object. Histograms bin width 20 ms. The ordinate is in spike/s. From Rizzolatti and Craighero 2004 (41).

Confirmation of this thesis appeared in a study conducted on human subjects in the same years (24). Fadiga stimulated the subjects' motor cortex (through TMS) and recorded the MEPs from their hand muscles while they 1) observed an experimenter grasping objects, 2) looked at the same objects, 3) observed an experimenter tracing geometrical figures in the air with his arm, and 4) detected the dimming of a light. Authors found that MEPs significantly increase during the conditions in which subjects observe movements. In addition, the MEP pattern reflected the pattern of muscle activity recorded when the subjects executed the observed actions.

Maeda and colleagues (36) further investigated the selectivity of modulation of cortico-spinal activity, testing subjects observing different finger movements in different hand orientations. Their outcomes slightly diverge from those observed in monkeys. The observation of a movement enhances motor output to the muscles involved in the movement, as previously demonstrated. Differently, they described a high degree of specificity of this observation-induced motor cortical modulation, depending on hand orientation. The modulation was maximal when the observed action corresponds to the orientation of the observer.

The observation-induced motor cortical modulation also appears to be time specific. The effects of different phases of an observed movement on the modulation of cortical motor output were studied by Gangitano and colleagues (26). In this study, a video-clip of a reaching-grasping action was shown and single TMS pulses were delivered during its passive observation. MEPs amplitude became larger with increasing finger aperture and became smaller during the closure phase, suggesting that the time course of cortical facilitation follow the phases of the observed action.

In 2008 Aglioti (1) noticed that the cortical facilitation induced by AO depend on subjective motor repertoire. Authors asked subjects to watch videos displaying basketball shots (in and out), soccer kicks or static images. Participants were divided in three categories: elite basketball players, expert watchers (journalists and coaches), and novices. TMS measurements were collected during the observation of such videos. Athletes and expert watchers presented an increase in motor excitability when they observed a basket action (same for shots in and out), rather than a soccer action or a static image (Figure 1.1.3.7).

However, a specific increase of motor facilitation for the hand muscle (involved in controlling the ball trajectory) was only found when basketball players observed out shots. The results suggested that mere visual expertise may trigger motor activation during the observation of domain-specific actions, but only elite players (who experienced an extensive motor practice) possess a fine-tuned motor resonance system.

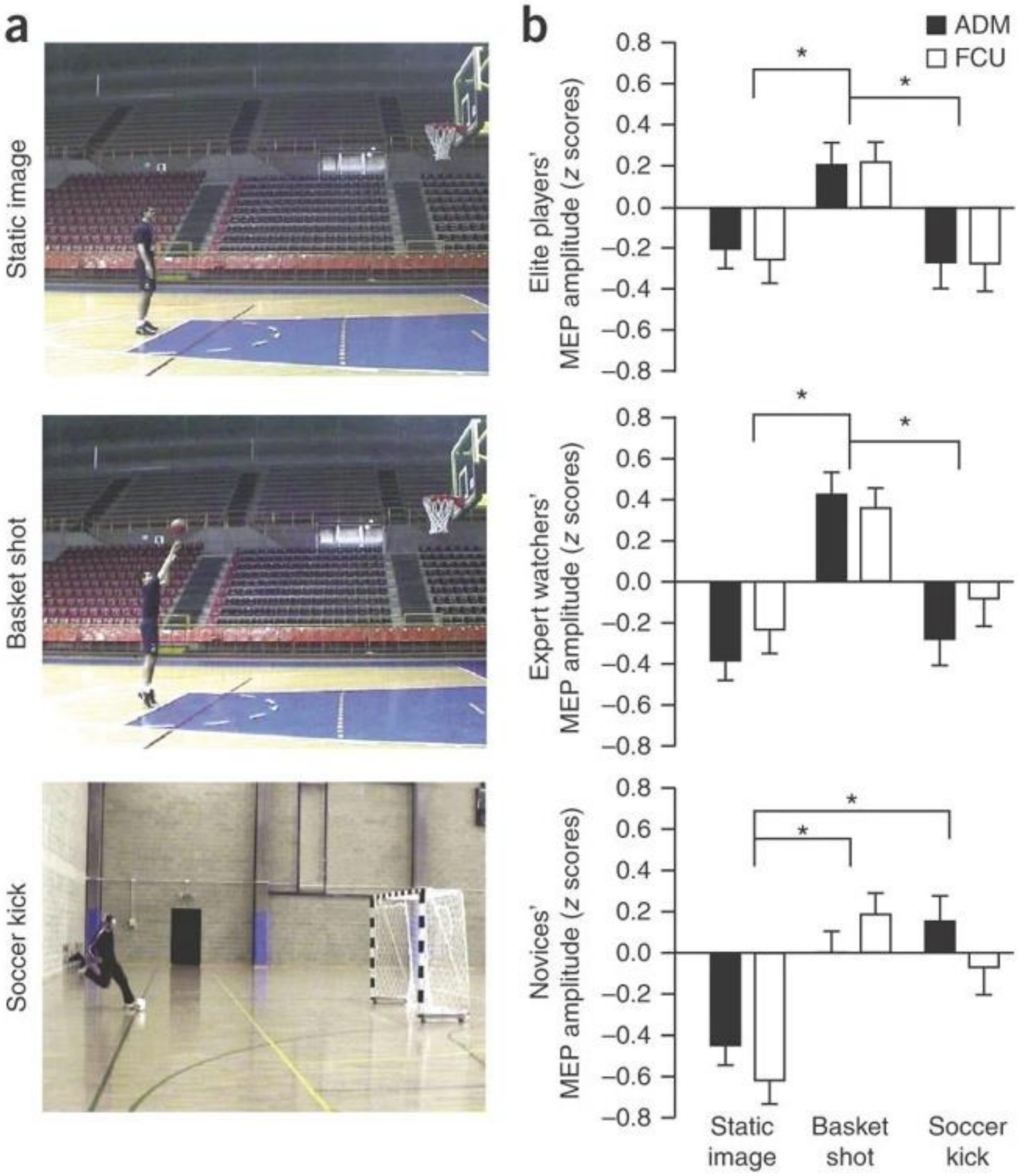


Figure 1.1.3.7 | a: Snapshots of three examples of static image, basket shot and soccer kick videos. b: MEP amplitudes (z scores) recorded from the ADM and the FCU in the three observation conditions (basket, soccer, and static image) for elite players (upper), expert watchers (centre) and novices (lower) groups. Error bars indicate standard errors. * indicate significant comparisons ($P < 0.05$) between the three observation conditions in each group. From Aglioti et al. 2008 (1).

fMRI studies are also in line with these findings. Calvo-Merino and colleagues (11) used fMRI to study differences in brain activity between watching an action that one has learned to do and an action that one has not, to assess whether the brain processes of AO are modulated by the expertise and motor repertoire of the observer. They recruited three groups of people with different acquired motor skills. Experts in classical ballet, experts in capoeira and inexpert control subjects viewed videos of ballet or capoeira movements. Authors compared the brain's activity of dancers watched their own dance style with the brain's activity of the same subjects when observed the other style. Results revealed that the influence of motor expertise on AO induce an increased bilateral activation in premotor cortex and intraparietal sulcus (in addition to right superior parietal lobe and left posterior superior temporal sulcus regions) when expert dancers viewed movements that they had been trained to perform compared to movements they had not.

One years later, the same group of authors (12) repeated the same experiment but distinguished, this time, male and female dancers. Dancers of both genders watched some ballets performed by only one gender. They found greater premotor, parietal, and cerebellar activity when dancers viewed moves from their own motor repertoire, compared to opposite-gender moves that they frequently saw but did not perform. As Aglioti, Calvo-Merino showed that the 'mirror system' integrates observed actions of others with the individual's motor repertoire, providing first evidence that the human brain understands actions by motor simulation.

Conspicuous number of studies investigating biological and non-biological movements could help to reinforce this affirmation. In a study by Saygin and his colleagues (47), participants' brain activity was monitored by means of fMRI technique during the observation of point-light biological motion and scrambled animations (the latter created by randomizing the starting position of the point-light while keeping the trajectories intact). The robust responses to biological movements were observed in frontal areas and indicated that these stimuli can also recruit AO networks, although they are very simplified and characterize actions by motion cues alone. The finding that even point-light animations evoke activity in frontal regions suggested that the motor system of the observer may be recruited to "fill in" these simplified displays.

Furthermore, Bisio et al. (8) showed that automatic imitation, namely one of the behavioural consequences of the motor resonance evoked by AO, disappeared when a uniformly accelerated motion, not belonging to the motor repertoire of a biological agent, moved a visual displayed dot, suggesting that the possibility of mapping the observed kinematics onto the internal motor repertoire is a requisite to evoking motor resonance. Similar results were obtained when the visual stimulus was a humanoid robot (7). The imitation process was equally evoked by the observation of a human demonstrator and by the observation of a humanoid robot, but only when it moved according to the biological laws of motion.

When switching in TMS studies the results seem more controverted. For example, Romani and colleagues (43) conducted a series of experiments examining the effects of observing biologically possible compared to biologically impossible movements (i.e., digits moving in an impossible range of movement) and showed that facilitation occurred in both conditions.

Craighero et al. (13) studied participants who observed a point-light display moving according to a biological and a non-biological law of motion and failed to find differences in primary motor cortex excitability between the two conditions.

On the other hand, Avanzino et al. (4) detected an increase in corticospinal excitability when participants observed a sequence of finger opposition with movements rate similar to their own rather than when looking at faster movements.

As motor imagery, the repetitive use of AO could induce cortical and behavioural changes. In 2015 Kirsch and Cross (34) tested a group of dance-naïve participants who learned a series of complex, whole-body movement sequences via an interactive dance video game. They probed how action representations change as types of experience combined, focusing on action learning in 3 modalities: auditory, visual, and motor. In the auditory group participants spent time each day listening to the soundtrack, in the visual group was added a layer of action information with visual cues of the specific movements associated with the music, and in motor group subjects combined physical, visual, and auditory experience. The subjects' learning was assessed by brain and behavioural measures. Results indicate that layering experience across these 3 modalities activates several sensorimotor cortical regions associated with AO network. When subjects learned with more modalities, they present a greater response in the AO network regions during action perception. Moreover, a correlation between left premotor activity and participants' scores for reproducing an action suggests that the better an observer can perform an observed action, the stronger the neural response is.

Other suggestion of brain plasticity and motor learning induced by AO were reported by Stefan et al. (49). He used TMS to show that observation of another individual performing simple repetitive thumb movements gives rise to a kinematically specific memory traced in the motor cortex. After observing a 30-min video showing thumb movements in a direction opposite to the baseline TMS-evoked direction, authors found an increased the probability of TMS-evoked thumb movements to fall within the observed direction. Furthermore, authors found an altered acceleration of TMS-evoked thumb movements along the principal movement axis and the balance of excitability of muscle representations active in the observed movements (in favour of the observed movement direction).

Contrariwise, Bisio et al. (4) reported no modifications motor cortical after AO training. Author assumed that to evoke plasticity in the primary motor cortex through AO is necessary combine it with afferent signals coming from periphery. Indeed, authors described an increasing in primary motor cortex excitability only after a protocol combining AO (observation of a video showing repetitive thumb index tapping movements) with peripheral electrical nerve stimulation and no cortical changes after protocols using only one technique (AO or peripheral electrical nerve stimulation).

Close results concerning behavioural measurements were announced by Bove et al. (10), showing a vanishment of AO influence if motor practice is not concurrent or immediately performed after it.

In this paragraph, we supplied a general view of two mental techniques widely used to enhance motor performance. AO and MI present very similar features that permit partially shifting the discoveries obtained from one technique to the other. Both produce significant neurophysiological and behavioural changes, suggesting that they are equally effective to produce motor learning. To maximise motor resonance's phenomena AO and MI must involve motor actions that people can understand and perform

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1.2 Experimental Contribution

1.2.1 Motor condition and cognitive processes: A pilot study on aerobic capacity and working memory in a real-life scenario

Introduction

Engaging in physical activity programs, in addition to common physical health benefits (37), was shown to elicit a wide range of neural changes (13).

For instance, at the molecular level, aerobic exercise was shown to induce changes in brain-derived neurotrophic factor, insulin-like growth factor 1, and vascular endothelial growth factor (11). At the cellular level, physical exercise stimulated gliogenesis, neurogenesis, synaptogenesis and angiogenesis (25). Physical activity also induced changes involving the white matter (30), with evidence of a protective (or even restorative) role of cardiorespiratory fitness and exercise against cognitive and neurobiological deterioration (36). Moreover, converging lines of research indicate that enhanced aerobic fitness may improve cognitive functions associated with executive control processes, such as planning, scheduling, inhibition and working memory (WM) (23). Among them, WM (i.e., the limited capacity storage system involved in the maintenance and manipulation of information over short periods of time (3)), plays a pivotal role in different higher-order cognitive functions, including learning (1) and tasks related to reading and language (5).

For this reason, identifying physical markers able to predict WM capacity along the students' lifespan (e.g., aerobic fitness) may help monitor the student population in order to adopt preventive actions to enhance brain health and offer equal learning opportunities.

Nevertheless, during everyday activities requiring cognitive abilities (e.g., school lessons, learning, social interactions), WM is not used in its “pure” form but is combined with multiple sources of variance (34). Therefore, tasks evaluating the multifaceted view of WM can be employed to better reflect a real-world scenario. Similarly, cardiorespiratory fitness could be assessed through direct measurement, by evaluating the maximal oxygen uptake (VO2max) attained during a laboratory-based incremental exercise test (32), or through indirect measurement. The latter can be easily performed using distance- or time-based field tests (26), which are widely used in real-life sports and educational settings (e.g., during school gym classes).

Recently, different studies suggested a relation between indirect measures of aerobic fitness and cognitive functions in clearly defined groups (e.g., children, young adults, elderly), but they were limited to specific age-related cognitive outcomes (14, 15, 19, 22).

In this context, it could be of interest to investigate the relation between aerobic and WM-related capacity in a sample of young healthy subjects during their whole school career (i.e., from primary school to university), known to be the major cognitive development period. In the current study we investigated the correlation between the VO2max, measured indirectly through the 12 minute-walk/run test (12m-WRT), and two WM-related tasks, based on different stimuli (i.e., auditory vs. visual) in order to detect potential differential effects of aerobic fitness on cognitive development.

Materials and methods

Participants

A required sample size of 40 participants was estimated using G*Power software, based on a desired power of 0.80, alpha of 0.05 and a medium-to-large effect size of 0.42. The effect size was assessed based on the study by Kao et al. (22), who compared working memory capacity with VO2max in children.

Forty healthy participants (21 males, 19 females, mean age 19.18 ± 6.18 years, range 10-24, Caucasian) were recruited for this study. All individuals did not have any recent history of injuries or symptoms limiting their performance at walk/run tests and they all gave written informed consent in accordance with the revised Declaration of Helsinki. The study was approved by the local ethical committee (San Martino-IST Hospital: 452REG2015).

Experimental protocol

Participants were evaluated in one single session, during which all cognitive and physical measures were collected. Cognitive assessment was carried out first, consisting of two repetitions of two cognitive tests, described below. A 5-minute interval between each test's repetition was included. In order to exclude learning effects, each repetition of the cognitive tests was preceded by familiarization trials as recommended in the tests' instructions (17, 27) and the mean value of the two repetitions of each test was considered. Subsequently, the 12m-WRT was performed in a nearby sports facility.

Outcome measures

In order to assess WM-related capacity we used two cognitive tests widely adopted in research and clinical practice: the Paced Auditory Serial Addition Test (PASAT) (17) and the Symbol Digit Modalities Test (SDMT) (31). Both tests are used to examine the information-processing speed and WM functions in healthy subjects and in different patients' populations (28, 33).

PASAT test was administered through an audio track, consisting of sixty-one stimuli applied at an interval of three seconds. Participants were asked to add each new digit to the one immediately prior to it, and the raw score was considered as the number of correct sums given (out of 60) in each trial.

The SDMT is a substitution task in which subjects have 90 seconds to pair specific numbers with given geometric figures using a reference key. In the current study, oral responses were collected and the total number of correct substitutions (ranging between 0 and 110) was considered as a raw score.

In order to adjust the results obtained at the WM-related tests to the years of formal education of each student, the raw scores of PASAT and SDMT were normalized accordingly to Amato et al. (2) as follow:

$$Normalized\ PASAT\ =\ raw\ score - 1.698 \times (education\ - 12.4)$$

$$Normalized\ SDMT\ =\ raw\ score - 1.029 \times (education - 12.4)$$

Normalized scores were used for the main results statistical analysis.

The 12m-WRT was performed on a running track with clearly marked distance: cones at a specific interval (every 100 meters) were placed to enable measuring of the space. After a warm-up (5 minutes), participants performed the 12m-WRT, consisting of covering the longest distance possible in 12 minutes. They were free to adjust their speed during the run (10, 38). Walking was allowed, but participants were encouraged to push themselves as hard as possible to maximize the distance covered. Total distance was used to estimate the VO2max according to Bandyopadhyay (4) as follow:

$$VO2max\ =\ (22.351\ x\ distance\ covered\ in\ kilometres)\ -\ 11.288$$

Participants' heart rate (HR) was measured using a HR monitor chest strap and a sensor (Polar H10, Polar Electro, U.S.A.). At the end of the test, we collected participants' HR and rate of perceived exertion (RPE) through the 10-point BORG scale (1=minimal perceived exertion; 10=maximal perceived exertion) to estimate whether the 12mWRT had been performed correctly (i.e., whether individuals maximized the distance covered). The test was considered correctly performed when HR and RPE reached values > 90% of those required during cardiopulmonary tests with incremental load (i.e., HR ≥ 90% of age-predicted maximum and RPE ≥ 8.5) (18).

Data analysis

Shapiro-Wilk test showed a normal distribution of all variables analysed.

Unpaired T-Test was performed to evaluate possible differences in VO2max between females and males. Subsequently, a bivariate correlation was run between VO2max and cognitive measurements in both males and females.

The main analysis consisted of bivariate correlations between age, VO2max, PASAT, and SDMT. Subsequently, two partial correlations between VO2max and raw data of PASAT and SDMT adjusted for age were performed.

To evaluate possible differences in either physical or cognitive performance among different education levels, we compared each measure (VO2max, PASAT and SDMT) among three groups defined by the individual school grade (primary school, secondary school and university) using a one-way analysis of variance (ANOVA). Subsequently, separated bivariate correlations were run for each school-grade level group to assess possible differences among them. For each of these correlations, post-hoc power calculation was measured. Statistical analyses were run using SPSS 23.

Results

All participants completed the evaluation session without reporting any adverse event.

The mean heart rate obtained at the end of the 12m-WRT was 180.6 ± 11.8 bpm, corresponding to 91.7 ± 6.5 % of the subjective maximal heart rate. The median value of RPE reported at the end of the 12m-WRT was 8 (range 7-10).

The raw score obtained at the PASAT was 39.69 ± 11.32 for primary school students, 42.82 ± 12.76 for Secondary School students, and 49.63 ± 8.16 for university students. Concerning the SDMT, the raw score was 53.30 ± 9.88, 62.67 ± 6.57, and 73.63 ± 10.86 for primary, secondary and university students, respectively. The mean values of VO2max were 38.96 ± 10.87 ml kg-1 min-1 for male participants and 28.13 ± 6.71 ml kg-1 min-1 for female participants.

Unpaired T-Test showed a significant difference between males and females ($T = 3.74$; $p < 0.001$). However, a significant correlation between VO2max and cognitive measurement in both males ($r = 0.521$, $p = 0.015$ for PASAT and $r = 0.573$, $p = 0.006$ for SDMT) and females ($r = 0.592$, $p = 0.007$ for PASAT and $r = 0.396$, $p = 0.042$ for SDMT) was observed. For this reason, we considered all participants together.

As a result of the main analysis, a significant correlation between VO2max and PASAT ($r = 0.442$; $p = 0.004$) and between VO2max and SDMT ($r = 0.454$; $p = 0.003$) (Figure 1.2.1.1) was found.

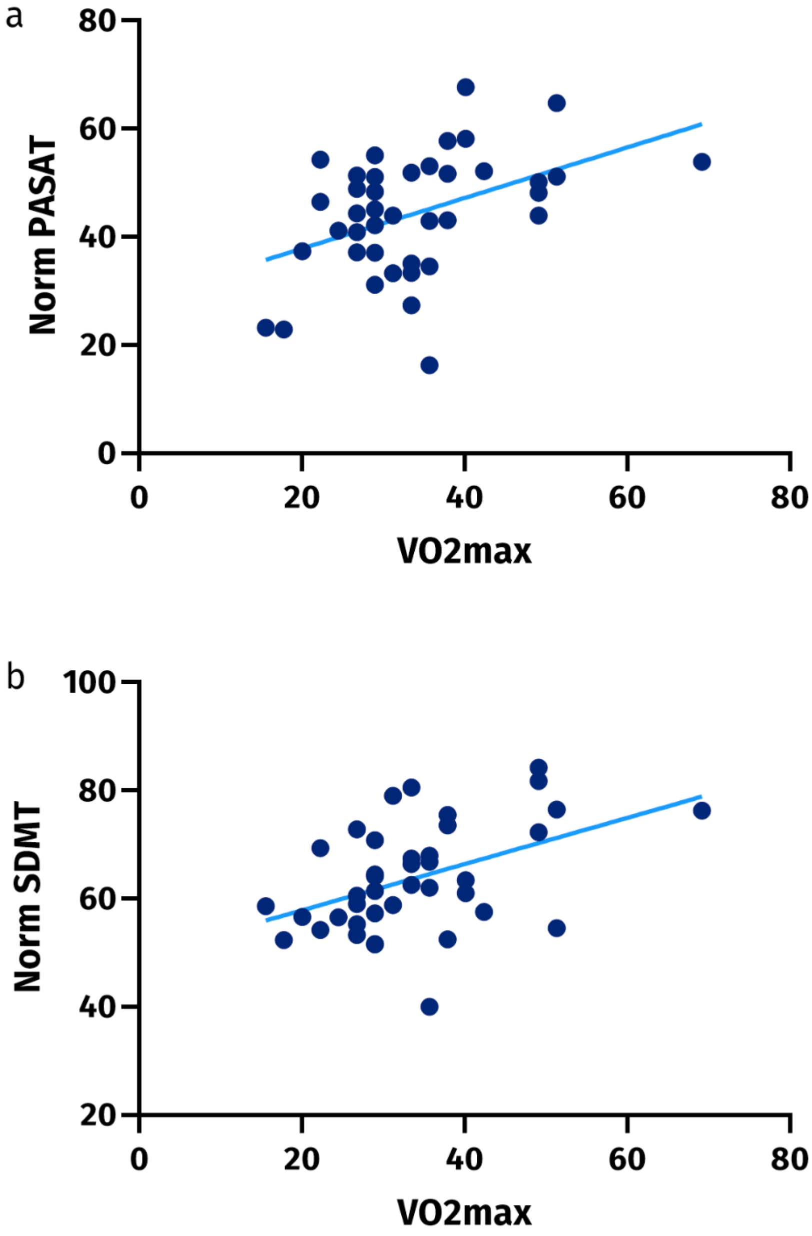

Figure 1.2.1.1 | Linear regression plot depicting the relationship between VO2max (ml kg-1 min-1) and a: the normalized paced auditory serial addition test (PASAT) score, b: the normalized symbol digit modalities test (SDMT) score.

No significant correlations were found between age and the cognitive tests, nor between age and VO2max. Furthermore, significant correlations between VO2max and raw data of PASAT and SDMT were maintained also after adjusting for age (PASAT: partial correlation = 0.446; p = 0.004; SDMT: partial correlation = 0.451; p = 0.004).

The ANOVA revealed no significant differences among school-grade levels groups with regard to VO2max, normalized PASAT score and normalized SDMT score. Table 1.2.1.1 summarizes the results obtained through the bivariate correlation analysis run for each school category. We found two different trends in the correlations of PASAT and SDMT in relation to years of formal education. In particular, aerobic fitness showed higher predictive values on WM-related performance investigated with PASAT in primary and secondary school student's groups. Differently, increasing predictive values of physical condition on SDMT performance were observed with the progressing of the school career.

Table 1.2.1.1 | Correlation between VO2max and the cognitive performance (evaluated by means of the paced auditory serial addition test - PASAT and the symbol digit modality test - SDMT) in the three groups according to the school-grade level.

	PASAT			SDMT		
	r^2	p	Power	r^2	p	Power
Primary	**0.612**	**0.003**	**0.974**	0.001	0.938	/
Secondary	**0.475**	**0.040**	**0.688**	0.207	0.219	/
University	**0.067**	**0.284**	**/**	**0.361**	**0.006**	**0.870**

Discussion

As expected, we found a difference on VO2max between males and females: males participants showed higher values of VO2max compared to females, in line with previous results (6).

However, both males and females showed a significant correlation between VO2max and PASAT as well as between VO2max and SDMT.

Overall, the results of this study indicate that higher levels of aerobic fitness are associated with greater performance in tasks that quantify the multifaceted nature of working memory

(WM) in both males and females, corroborating the hypothesis that increased cardiorespiratory capacity can lead to improvements in the cognitive control of WM-related capacity (21). The current results also suggest that the 12 minute-walk/run test (12m-WRT) is associated with cognitive performance measured trough PASAT and SDMT. In particular, PASAT score was highly predicted by the 12m-WRT in primary and secondary school students, whilst higher predictive values of the 12m-WRT for SDMT scores were found in the university population. These two opposite trends can be explained by the tasks' different modalities related to the individual aerobic capacity. In particular, in the PASAT, participants have a limited period of time to give the correct response, whilst the SDMT is a self-paced task. This may imply that the tests have different sensitivity to information processing speed. Moreover, it has been shown a detrimental effect of the auditory stimulus on accuracy and reaction time in paced serial addition tests in a study comparing two possible versions of stimuli administration: auditory (PASAT) and visual (PVSAT) (29). The authors suggest that the lower performance obtained at PASAT can be ascribed to the "interference effect" produced by the conflict between the stimulus input and the response output, corroborating the hypothesis of a higher engagement of information processing speed during PASAT.

Furthermore, differences between the administration of auditory and visual stimuli were also observed in cortical activity: specifically, the anterior cingulate and insular cortices were significantly influenced by modality, showing more pronounced activation during auditory testing (16).

As previously introduced, these factors seem to be differently influenced by physical activity according to age. Indeed, previous studies showed that physical exercise may play a crucial role in enhancing information processing speed and interference control, especially in the early phases of neurocognitive development (8, 20). Moreover, some authors suggested that aerobic fitness may play an important role in modulating the efficiency of neural networks implementing cognitive control in preadolescent children (19, 35). In a study based on electroencephalography measurements during a cognitive task in high- and low-fit children and adults, a higher association between fitness and neuroelectric indexes of attention and WM was found in children. This indicates a larger population of neurons being recruited in this age group (19). Furthermore, physical activity was demonstrated to promote the development of different brain structures in childhood, including those mainly activated during auditory testing, e.g., the anterior cingulate cortex (35).

This evidence could explain why the correlation found between VO2max and PASAT was more significant among primary and secondary school students. However, the correlation decreased when the groups' age increased: this could be due to the inter-stimulus interval used in the current study to allow all participants to perform the test (i.e., 3 seconds), which could be adequate for children and adolescents, whilst could be less difficult for university students, leading this group' results to a ceiling effect (12, 39).

Conversely, the strong correlation between VO2max and SDMT observed in university students could be ascribed to the higher validity, reliability, and sensitivity of SDMT, compared to PASAT, to determine WM function in healthy and cognitively impaired adults (7, 24).

Notably, we did not find any direct relationship between age and normalized scores of cognitive performances. This is in line with previous findings showing that aerobic fitness is the feature most related to academic achievement in pre-adolescents compared to other variables such as age, muscle strength, body composition and socioeconomic status (9).

Our results also suggest that the measures here adopted can be performed during childhood and youth to evaluate the relationships between physical and cognitive domains, paying particular attention in choosing WM-related tasks according to the school grade.

Future studies involving larger sample sizes are needed to generalize our results, to investigate further the correlations we found between cohorts and to test their statistical representativity. Further research is also required to explore the correlation between the 12m-WRT and WM in cognitively impaired people, to evaluate the effect of an intervention based on aerobic training in this population prior to recommend it as neurorehabilitative or as a neurorestorative activity.

In conclusion, the finding of the current explorative study provide evidence that the 12m-WRT is associated with WM-related performance in young healthy subjects and could become a recommended tool to monitor aerobic fitness in schools and to adopt preventive actions to enhance brain health and learning abilities.

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1.2.2 Executive function and motor performance: A pilot study on the application of motor imagery as a learning method during physical education lesson in high school

Introduction

Motor imagery (MI) is the mental simulation of an action without its actual execution (36). A large body of evidence supports the existence of a functional equivalence between MI and movement execution (ME) (9). This equivalence appears when comparing the response of the autonomic system (17), but also cortical and sub-cortical brain activations (13, 22, 30) during MI and ME. Furthermore, at behavioral level several studies has applied the mental chronometry paradigm and have showed that in case of simple gestures the duration of real and of imagined movements corresponded, in compliance with the isochrony principle (7, 18, 19).

On these bases, motor imagery has been successfully applied as motor learning technique (5, 8, 9, 20, 43). Moreover, it has been suggested as promising add-on to physical therapy in facilitating motor recovery in neurological patients (1, 26, 35), and to the conventional training in different sport domains for improving motor performance (9, 28).

Dedicated literature showing the beneficial effects of motor imagery on motor performance dates back to the mid-1930s (44, 45). In practice routines imagery is a well-established technique among sport experts (34, 37). Several studies showed the effectiveness of MI in connection with movement execution in sports requiring individual performance involving strength and flexibility, not to mention activities such as tennis and golf (14, 32, 39, 50). MI potential were also shown in team sports, examples being volleyball, basketball and soccer (2, 3, 11, 48, 49).

In addition, motor imagery has been applied as an education technique in medical field. Medical and nursing students, as well as resident staff with limited experience in surgery, were recruited for studies testing the effect of MI on a medical serve. Schuster and colleagues (46) summarized the results of these studies and concluded that MI is a valuable educational technique to be implemented as method in the clinical domain - also proposed by a more recent review (4). Although in sports, possessing a high level of experience seems to increase MI efficacy (41), in education most studies were aimed at students with limited or no experience in the requested performance. The positive results obtained by the latter point to the possibility of adopting motor imagery as an educational tool in schools.

Given these positive results as reported in sports and education literatures, motor imagery could be hypothesized as an appropriate tool to increase learning effectiveness as part of physical education lesson at school.

Furthermore, motor imagery technique complies properly with the European Council Recommendation (released on 22 May 2018, 2018/C 189/01) dealing with key competences for lifelong learning. One of the eight key competences is the “Personal, social and learning to learn competence”, which includes the ability to reflect upon oneself and effectively manage time and information, to identify one’s capacities, to focus and deal with complexity and to reflect critically and make decisions. Motor imagery might promote the development of this ability since it helps people to concentrate on complex tasks and to find solutions to complete such tasks - taking both internal and environmental information as a starting point. A potential consequence, therefore, of the application of MI to a physical education lesson might be an improvement in self-awareness, as suggested by studies on body perception and body schema (6), and by works showing a superposition between the frontoparietal networks active during MI and supporting awareness (15, 38, 47). Despite such potential, as far as is known, no study has yet tested whether MI could be applied alongside motor practice in a school environment.

The aim of this pilot study has been to test the feasibility and effectiveness of the application of motor imagery as a learning method alongside conventional teaching techniques founded on verbal instruction and movement execution during physical education lessons at high school.

Materials and methods

Participants

Fifty-four students in their first year at an Italian high school volunteered to participate in this study. They were divided in two classes of 26 (10 males and 16 females, mean age ± SD = 14.12 ± 0.33) and 28 students (9 males and 19 females, mean age ± SD = 14.19 ± 0.41). The first was designated as the Motor Imagery Group (MIG), and the second one as the Control Group (CG). Written informed consent was obtained from all participants and legal guardian before data collection. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of the University of Genoa (Comitato Etico per la Ricerca di Ateneo, n° 2020.1).

Experimental protocol

The experiment lasted 10 weeks (Figure 1.2.2.1) and was effected as part of the physical education lesson, timetabled once a week. At the first lessons, a dedicated experimenter cooperated with the teacher to explain the aim of the project and performed baseline evaluations (PRE). From week 2 to week 9 students took part in the training sessions, scheduled for the first 15 minutes and conducted by the experimenter in collaboration with the teacher. For the rest of the double period (1 hour and 45 minutes) the teacher proceeded with his syllabuses, not comprising the topic of the experimentation. The tenth lesson was dedicated to the experimenter's evaluations (POST), enabling assessment of possible differences from PRE evaluations. Evaluation phases and training sessions took place in a gymnasium.

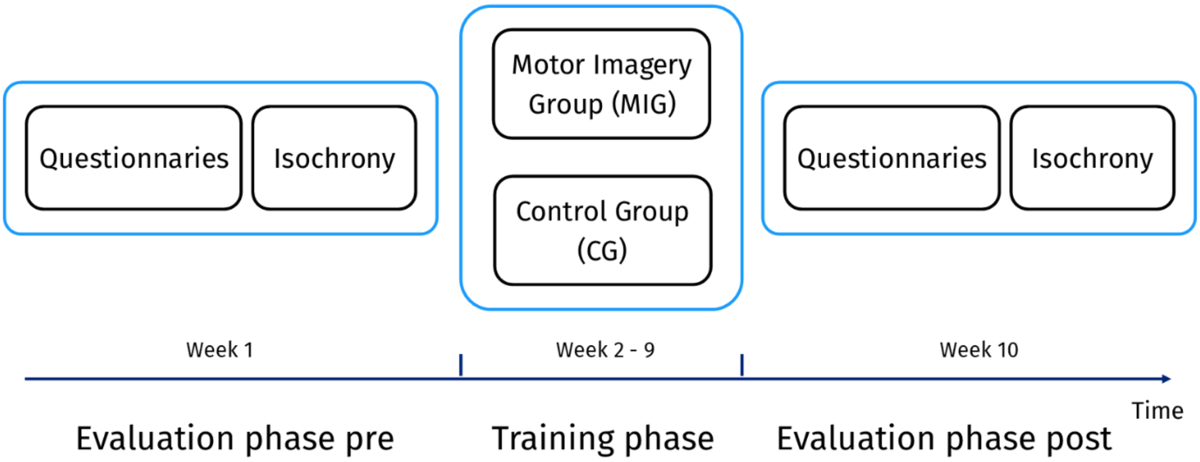

Figure 1.2.2.1 | Illustration of experimental design: During the first week of the experimentation (Week 1), the experimenter performed the evaluation phase (PRE). Questionnaire on motor imagery ability (MIQ-R) and enjoyment of physical education lesson (PACES) were administered. After that, participants performed the underhand serve (3 times) and imagined the same gesture. The isochrony between imagined and real movement and the number of ball that passed over the net (performance outcome) were computed. From Week 2 to Week 9, the two classes either performed the conventional training based on movement execution (Control Group) or added to the conventional training a motor imagery training (Motor Imagery Group). During Week 10, the experimenter replicated the same tests administered during week 1 (POST).

Evaluation Phase

Student assessment covered different kinds of evaluations, all of them conducted by the experimenter.

Questionnaires

In the beginning, each student received two questionnaires, each on a separate sheet. Students sat in a circle on the floor with the expert positioned in the center, from which position the first questionnaire instructions were read. The students were required to provide a written answer to each point of the questionnaire. Once they had completed the operation, the expert collected the papers with the answers to the first questionnaire and then proceeded in like manner for the second.

The first questionnaire was the Italian version of the Movement Imagery Questionnaire (MIQ-R) (33) - designed to evaluate the ability to form kinesthetic and visual images. MIQ-

R is an 8-item self-report questionnaire, in which participants rated the vividness of their mental representations using two 7-point scales (associated to visual and kinesthetic imagery): 1 corresponds to “really easy to feel/see” whereas 7 corresponds to “really difficult to feel/see” (best score = 8, worst score =56). Participants completed this questionnaire for PRE evaluation exclusively in order to assess their motor imagery ability - this latter considered good and comparable between groups (mean ± SD; MIG: 19±1.8; CG 19.5±1.8, t=0.19, p=0.85).

The second questionnaire was the Physical Activity Enjoyment Scale (PACES) (revised version by (40)), modified and translated into Italian (51), and specifically focused on physical education lesson - the aim being to test whether introducing MI technique changed perceived enjoyment of the lesson. PACES comprises a 16-item scale that assesses enjoyment for physical activity by asking participants to rate “how you feel at the moment about the physical activity you have been doing” using a 5-point Likert scale, from 1 (I totally disagree) to 5 (I totally agree).

Isochrony and performance outcome

The objective of this test was to evaluate the correspondence between the duration of real and imagined movements and the number of balls that passed over the net, bouncing into the opposite court (NBN). Concerning movement duration, the hypothesis was the lower the difference between one duration and the other, the more imagined movement (motor imagery - MI) resembles the real movement (movement execution - ME). Then, an increase in NBN after the training period would be an index of enhanced performance.

At the beginning of this session, the teacher explained the basic technique of the underhand serve and provided a brief video-tutorial. The video was the same for both classes. After the teacher’s explanation, students were required to draw up so as to act in turns just beyond the edge of the volleyball court. Once the correct posture had been adopted, they had to carry out three underhand serves. The experimenter used a chronometer to measure the time lapse between the first movement the student started in preparation to serve and actual striking of the ball. The time taken and technical success of the serve (NBN) were recorded on paper. In the end, again in turns, each student was required to take up his position once again, and to imagine the same action three times. During MI participants were required to perform a kinesthetic imagery (i.e., to focus on the sensory information generated during the actual action execution, including the strength and effort (16)) of the action previously carried out. Further, they were instructed to say “start” when starting to imagine the movement and “stop” when their hand struck the ball virtually. The experimenter once again employed a chronometer for MI durations and took note accordingly. In the evaluation phase, participants were asked to focus exclusively on their own movement, and not on the ball movement, in order to create a comparison between imagined movement and real movement duration.

Training Session

Motor imagery group (MIG)

Students carried out three underhand serves during each session. They were positioned in three rows just beyond the edge of the volleyball court - in the left corner, the right corner and the center. All at the same time, three students went through the procedure of imaginary and then real underhand serve. Once serving had been accomplished in their starting line, each student moved away so as to take up a position in one of the other lines. In this way each student executed the movement in each of the three positions. Before engaging in any movement, they were instructed to firstly imagine the gesture. During training, the solution adopted was combined kinesthetic imagery, when the focus was on oneself, and visual imagery, when the focus was external on ball trajectory. When motor imagery technique is applied in laboratory setting, it is common to focus on only one modality (5, 7, 12). Conversely, during practical applications in sport the modalities, as well as the focus, are less specific - thus, affording the athletes the possibility to pay attention to different aspects of the action (11, 14). The specific instruction was: “*Imagine the underhand serve. Focus on the sensation you feel during the actual execution, and then visualize the ball that gets over the net and falls inside of the opposition court*”.

Control group (CG)

Students in the control group carried out the same training as those in the MIG - except for MI before the serving action. Typical learning methods during physical education lesson did not demand intervention other than the repetition of the action. This is why it was decided to not add placebo manipulation to conventional training - avoiding possible confusing effects.

Data Analysis

The evaluation of the two types of training was performed by comparing the PACES score, the duration of real and imagined movements (mean values obtained over the 3 serves), and the NBN (computed as the sum of the 3 serves), before (PRE) and after (POST) training. The isochrony index (s) was calculated as the difference between the mean duration of imagined and real movement. A difference equal to zero yields perfect isochrony between the two movements.

Normality was checked by means of Shapiro-Wilk tests. PACES score, durations of real and imagined movements, and the isochrony index were normally distributed.

PACES score and the isochrony index were analyzed by means of ANOVAs with TIME (2 levels, PRE and POST), as within-subject factor, and GROUP (2 levels, MIG and CG), as between-subject factor.

The statistical analysis on durations of real and imagined movements was performed by means of an ANOVA with TIME (2 levels, PRE and POST) and MOVEMENT (2 levels, ME and MI), as within-subjects factors, and GROUP (2 levels, MIG and CG), as between-subject factor.

The NBN values were not normally distributed. In order to compare the two groups at PRE and POST Mann-Whitney tests were applied, while to test the difference between PRE and POST in each group Wilcoxon tests were used.

In both groups Spearman’s correlation was used to evaluate the relationship between the percentage changes in the isochrony index, computed as (isochrony indexPOST- isochrony indexPRE) * 100, and the differences between the number of ball that passed over the net (NBNPOST-NBNPRE). The lower the isochrony percentage change, the better the improvement of the isochrony from PRE to POST. The higher (NBNPOST-NBNPRE), the better the performance outcome from PRE to POST.

All the statistical analyses were performed by means of Statistica.

Results

The The experimentation comprised 54 students. Unfortunately, the analysis concerned only 32 students (16 in MIG and 16 in CG). Some students' data were removed because of absence from one of the evaluation phases; others were not considered because they participated in fewer than 6 lessons out of 8. Power of sample was calculated using a posteriori analysis by means of G-Power software, which showed good results for isochrony index; with η2=0.17 (see results of ANOVA on isochrony index), effect size= 0.45, 1-β=0.99.

Mean values of all parameters are reported in Table 1.2.2.1.

Table 1.2.2.1 | Mean values (± standard error) of parameters used to evaluate changes before (PRE) and after (POST) the training in the motor imagery group (MIG), who added motor imagery (MI) to movement execution (ME), and in the control group (CG), who performed only ME.

	MIG		CG	
	Pre	Post	Pre	Post
PACES score	3 ± 0.1	3.06 ± 0.1	2.91 ± 0.08	3 ± 0.1
Movement Duration (s)				
ME	0.52 ± 0.03	0.6 ± 0.04	0.65 ± 0.06	0.61 ± 0.05
MI	1.69 ± 0.14	1.62 ± 0.14	1.85 ± 0.19	1.89 ± 0.15
Isochrony index (s)	1.17 ± 0.13	1.02 ± 0.14	1.20 ± 0.19	1.28 ± 0.15
NBN	1.94 ± 0.28	1.90 ± 0.27	1.94 ± 0.27	1.88 ± 0.24

The statistical analyses on PACES score did not reveal significant differences between the two evaluation epochs and between the two groups, nor any significant interaction between TIME and GROUP.

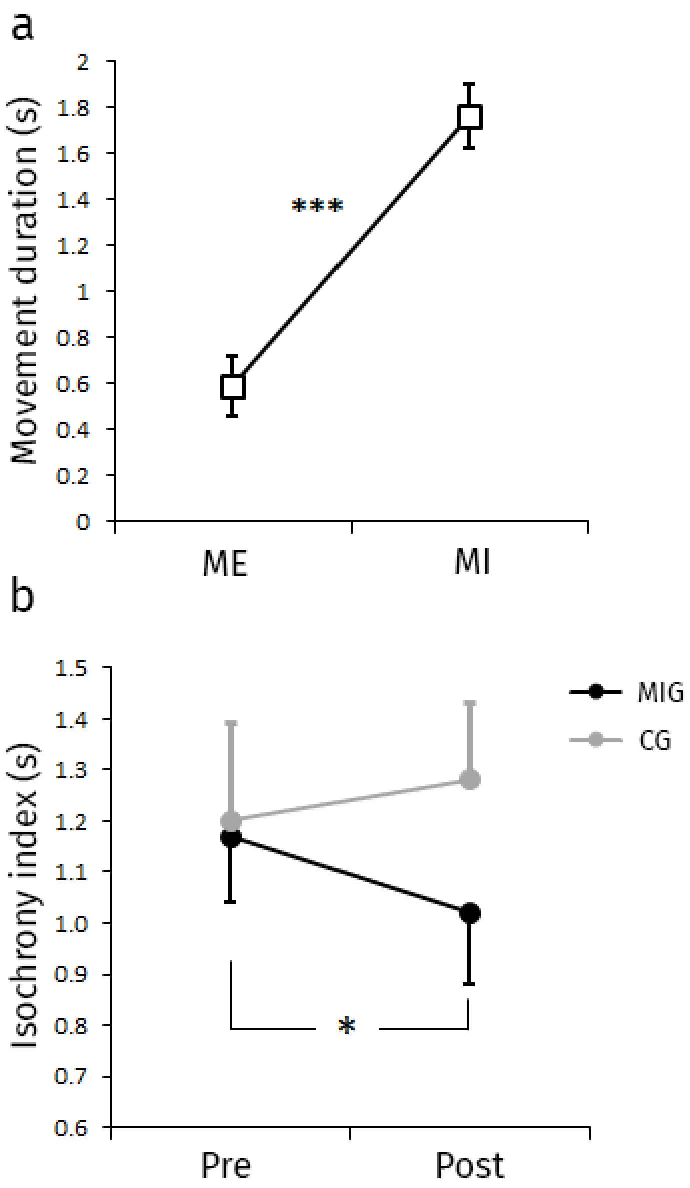

Figure 1.2.2.2 | (a) Mean values of the duration (s) of executed (ME) and imagined (MI) movements. (b) Isochrony index mean values of the Motor Imagery Group (MIG—black circles) and Control Group (CG—gray circles). Error bars indicates standard errors. *p < 0.05, ***p < 0.001.

The comparison between real and imagined movement duration showed a significant effect of the factor MOVEMENT ($F_{(1,30)} = 115.67$, $p < 0.0001$, $\eta^2 = 0.79$), indicating a significantly higher duration of the MI vs ME (Figure 1.2.2.2 a).

The analysis on the isochrony index showed a significant interaction between TIME and GROUP ($F_{(1,30)} = 6.26$, $p < 0.05$, $\eta^2 = 0.17$). The Newman-Keuls post hoc comparisons revealed a significant decrease in the isochrony index value of MIG after the training period (p = 0.02) (Figure 1.2.2.2 b).

The analysis on NBN did not show any significant difference between groups both at PRE and at POST; nor were there any significant changes between PRE and POST in the two groups.

Spearman's correlation between (NBNPOST-NBNPRE) and the isochrony percentage change was significant for MIG (R = -0.50, p = 0.046) and showed that participants who improved NBN after MI training were those who showed a higher isochrony after the training than before. No significant correlation was observed in CG (R = -0.26, p = 0.33) (Figure 1.2.2.3).

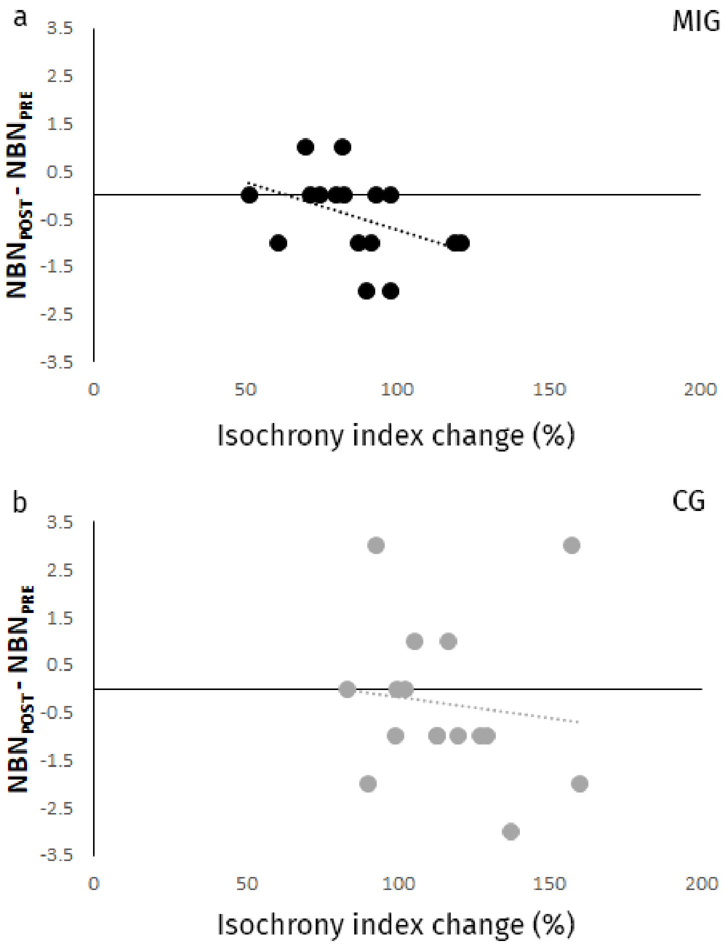

Figure 1.2.2.3 | Relationship between the difference from baseline to after the training period in the number of ball that passed over the net (NBNPOST-NBNPRE, y-axis), and the percentage changes in isochrony index (x-axis) in Motor Imagery Group [MIG, (a)] and Control Group [CG, (b)]. Each circle represents the data of a one participant.

Discussion

The aim of this pilot study was to test the feasibility and the effectiveness of a new teaching program that combines movement execution with motor imagery, as applied to physical education lesson in a high school context.

The results showed no differences between motor imagery and control groups on perceived enjoyment of the lesson (PACES score). Imagined movement duration was significantly longer than that of real movement - irrespective of the group and evaluation epochs. Furthermore, the isochrony index - a parameter yielding information on similarity between imagined and real movement – was computed. The results showed a significant decrease in index value after the training period exclusively in the motor imagery group, indicating that the duration of the imagined movement approached the duration of the real movement in MIG but not in CG. As regards performance index, the number of balls that passed over the net was counted. No changes appeared in this parameter in either group after the training. A significantly negative relationship appeared between the percentage change in the isochrony index and the differences between NBN from baseline (PRE) to the end of the training period (POST).

The application of motor imagery has by this stage become widespread in neuroscientific literature, where the majority of studies published on this matter show an improved motor performance after training based on MI (for instance (21, 23–25)). The improvements did not focus merely on behavioral response but also on plastic modifications to the cerebral activity, similar to those to be observed after physical practice (5, 42).

In the field of sport, and specifically in volleyball, Afrouzeh and colleagues (2) showed that volleyball beginners, who combined physical practice and the PETTLEP model (i.e., a model providing a framework for the effective execution of motor imagery use) improved their “passing” abilities more than the other groups. Here, the focus has been on the MI effect on another gesture in volleyball - the underhand serve, one of the first basic skills acquired by beginners. Motor imagery technique is often used by elite athletes (70–90% reporting they use MI for improving their performance (34, 37)), who mastered the technical skills of their sport. Nevertheless, beginners too might benefit from such application, as shown in the seminal study on piano performance by Pascual-Leone and Coworker (42), and in studies published in educational literature (46). However, the lack of experience in volleyball might explain the lack of isochrony between real and imagined movements; the duration of imagined movements was significantly higher than that of real movements in both groups and testing epochs. Nor was this the first study showing the absence of isochrony between real and imagined movements. In a study on motor imagery performance in tennis players and gymnasts, Guillot and colleagues (29) found that the duration of the imagined movements was higher than the case of executed movements. To explain this result the explanation was offered that image accuracy would appear to be a more important factor than the temporal characteristics of the movement, leading to more time dedicated to imagining the action than to executing the same. In the present study, the increase in time devoted to MI vs ME might have been caused by the difficulty the participants experienced in creating a kinesthetic imagine of this new action. Participants, therefore, might have needed more time to imagine the action accurately. Nevertheless, whether the expertise level alters/facilitates the temporal equivalence between executed and imagined movement duration remains to be determined in future studies (31). Another factor that might have altered the isochrony is the actual time of the day (the morning) when the test and the training took place. It was shown that isochrony changed throughout the day, depending on the circadian rhythm - maximum between 2 and 6 p.m. (27). Unfortunately, the high school timetable had already been established before experiment inception and it was not possible to alter it.

In face of this result, however, the present study showed that the difference between imagined and real movements, as it appears on the isochrony index, evolved differently in the two groups. In the control group no differences emerged before and after conventional training, whereas in the motor imagery group the value decreased significantly after protocol administration. Changes in isochrony index might indirectly point to possible changes in the cortical sensorimotor representation of the movement. For this reason, a suggestion is that improvement in isochrony might be a consequence of an improved ability to gather the sensory information required to plan the movement. Such information might comprise visual information concerning the volleyball court, proprioceptive information concerning the posture the student has to adopt in the different phases of the action, and the strength the student has to channel to the ball so that it might pass over the net, at the same time respecting the area of the opposing court. This improvement might also result from facilitation in creating the motor plan to complete the movement. These results are signs of the occurrence of motor learning and are at the basis of a better motor performance. The significantly negative relationship found exclusively in the motor imagery group between improvements in isochrony and in performance before and after training supports this hypothesis. Participants showing improvements in isochrony after MI training (isochrony index % change < 100) were students showing higher success in performing underhand serves (NBN > 0) or students who had not worsened (NBN = 0).

Improvements in the mental representation of the movement, therefore, enhanced/preserved the effectiveness of the volleyball serve. However, it has to be acknowledged that motor imagery might have been a strong source of motivation for the experimental group, leading its participants to achieve better concentration during testing procedure. The improvements in isochrony, thus, might not be ascribed merely to the neurophysiological effects of motor imagery, but also its motivational role.

No improvements whatsoever were recorded in NBN - considered as an index of performance. An explanation might lie in the limitations of the present study. A possible drawback was the limited time devoted to the experiment (about 15 minutes per lesson for a total of 8 weeks), structured to interfere as little as possible with the activity already planned for the physical education lesson proper. Such a time span might not have been sufficient to improve the motor performance. Indeed, it is worth noting that improvements occurred neither in the control nor in the motor imagery groups. To highlight such an issue, it would have been useful to devise a questionnaire at the end of the training period to ascertain how training was executed and how was participants' experience about that. Answers would have helped to infer possible criticisms explaining the lack of skill enhancement.

In specific reference to the role of motor imagery, the following hypothesis cannot be underestimated; lack of volleyball experience might have decreased the quality of the imagined movement and, consequently, its effectiveness. Furthermore, MI quality might have suffered because of the time of day training was scheduled, as has already been mentioned before.

Future studies dealing with this matter might, therefore, be organized so that motor imagery testing and training sessions are held in the afternoon and along with motor imagery as a learning method employed throughout the entire lesson.

Finally, a possible variability was introduced by the experimenter with chronometer employment to assess movement duration. Since this study was a preliminary test to assess the feasibility of the application of this procedure at school, the same study had to make do with the technological means at the school's disposal.

In conclusion, the present pilot study is the first to test the feasibility and the effectiveness of the application of motor imagery as add-on technique to conventional learning methods for physical education lesson at high school. As regards feasibility, the students positively adhered to the program and were curious about this new method. The majority of the participants from the motor imagery group anecdotally reported that they learnt to concentrate explicitly on the action and to imagine the same before accomplishing. This paper’s proposal is that future experimentations might be designed so as to integrate motor imagery throughout the entire lesson and for a longer period of time, thus further assessing its potential in performance enhancement. Despite above mentioned limitations, results are encouraging in term of the improved sensorimotor ability shown by the motor imagery group and not by the control group. The conclusion is that this study embodies the first proof of concept aimed at introducing motor imagery as educational method for schools and might be considered an example in didactic innovation.

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1.2.3 Motor fatigue and executive function: Relationship between muscle fatigue and temporal expectation

Introduction

Accurate temporal estimation is an essential feature to face the variety of situations and gestures of our daily life, which always requires precise time keeping (27). To solve this task, people extract patterns of temporal regularity in the events (4). This ability, referred as temporal expectation, appears to be particularly important for sportive competition, given the key role of action anticipation, motor coordination and motor synchronization for a successful performance (26).

The ability of temporal expectation has long been known to improve the ability of action preparation and execution (19), as well the long-term sport training is related with the superior ability to extract accurate temporal predictor from visual stimuli (26). This phenomenon seems to be strongly related to the sports experienced by subjects (6). Indeed, athletes engaged in specific sports practice are more accurate to predict the outcome of the task which they are familiar with. This difference could be explained taking into account subjective abilities to extract body kinematics and mentally simulate the not visible or not yet happened parts of action (1, 3, 6, 15).

The gained experience likely influenced the subjective sensorimotor representation of the acquired motor ability, and some studies suggested that temporal expectation relays on an internal action simulation, based on that representation (23, 32). This hypothesis is supported by the existence of an action-observation matching system, that automatically

maps the observed events into the subjective motor repertoire (11, 13), thus responsible of a representational overlap between the sensory afferences and the motor plans (8, 16).
Evidence of this theory are reported from Stadler and colleagues (25), who found an increase in premotor area activation when observers predicted the future course of partly invisible action sequences in which they are experts, corroborating the hypothesis that a good internal action representation could increase the precision with whom the observed actions are predicted.

When people constantly use a specific body part, the brain reorganize itself to promote an enlargements of the body part's motor cortex representation (29). The number of dendritic spines grew in both superficial and deep layer motor cortex neurons, including in corticospinal neurons (10).

These changes in motor cortex could lend modification in motor recruitment strategies resulting in adaptation related to preferential use. For example, Adam and colleagues (2) observed that the dominant hand possesses unique neuromuscular properties with respect to non-dominant hand. Similar factors were also found in muscles performing better during endurance tasks (2, 28), probably related to different motor strategies adopted granting a cleaner motor control.

Consequently, became experts in specific actions cloud allow us to better stimulate it in our mind but also to maintain efforts involving specific body parts longer.

A common condition known to alter the sensorimotor representation, as well the motor performance of a certain action, is muscle fatigue.

Muscle fatigue is defined as the decline in ability of a muscle to generate force consequent of vigorous exercise (12). In addition to well know alteration on force generation, muscle fatigue also affects movement coordination (22) and alters movement or effort senses (30). Demougeot and Papaxanthis (7) observed that muscle fatigue drastically modifies the temporal features of both mental and actual movements. In this experiment, participants performed (mentally and actually) a sequence of vertical arm movements before and after a fatiguing exercise involving the right arm. The authors found similar durations for actual and mental movements before fatigue, but significant temporal discrepancies after fatigue whilst immediately after fatigue in isochrony was lost. These results underline the interdependence of motor and cognitive states and suggest that mental actions integrate the current state of the motor system.

To explore this hypothesis our study has the primary aim of evaluating the effects of muscle fatigue on a temporal expectation task. The secondary aim was to establish the role of temporal expectation ability on the time to exhaustion performance. To do that we induced quadriceps femoris' muscle fatigue and tested the temporal expectation ability through a task showing an action in which lower limb muscles are involved.

Materials and methods

Participants

Thirty-four healthy young adults (mean age 23.5 ± 2.1 years, 20 male and 14 female) were recruited from the University of Burgundy volunteered to participate in this study. Twenty of them (mean age 23.3 ± 2.4 years, 11 male and 9 female) participated in the main experiment and the others fourteen (mean age 23.8 ± 1.5 years, 9 male and 5 female) were recruited in a control experiment. All participants had no recent history of injuries or symptoms that can limit the physical performance, such as surgery or injury to lower limbs structures (tendons, ligaments, etc.), any history of neurological disorders, and a normal or corrected to normal vision. All participants were given oral instructions to sleep for the last 7 h and avoid any vigorous exercise the day before visiting our laboratory.

The regional ethics committee of Burgundy (C.E.R.) approved the experimental protocol, which was performed in agreement with local requirements and international norms (Declaration of Helsinki, 1964).

Main Experiment

Study Design

For the main experiment, we employed a single-blind, randomized, counterbalanced experimental design. The subjects came to the laboratory twice. During both visits, subjects performed a temporal expectation task before (Pre) and after (Post) one of the two possible experimental conditions. Condition order was randomly assigned according to balanced permutation.

A maximal voluntary isometric contraction (MVIC) was measured before (Pre), immediately after (Post_0) and five minutes after (Post_5) the conditioning to assess its effect on muscle fatigue and to monitor the recovery. Figure 1.2.3.1 summarizes the timeline of the experiment.

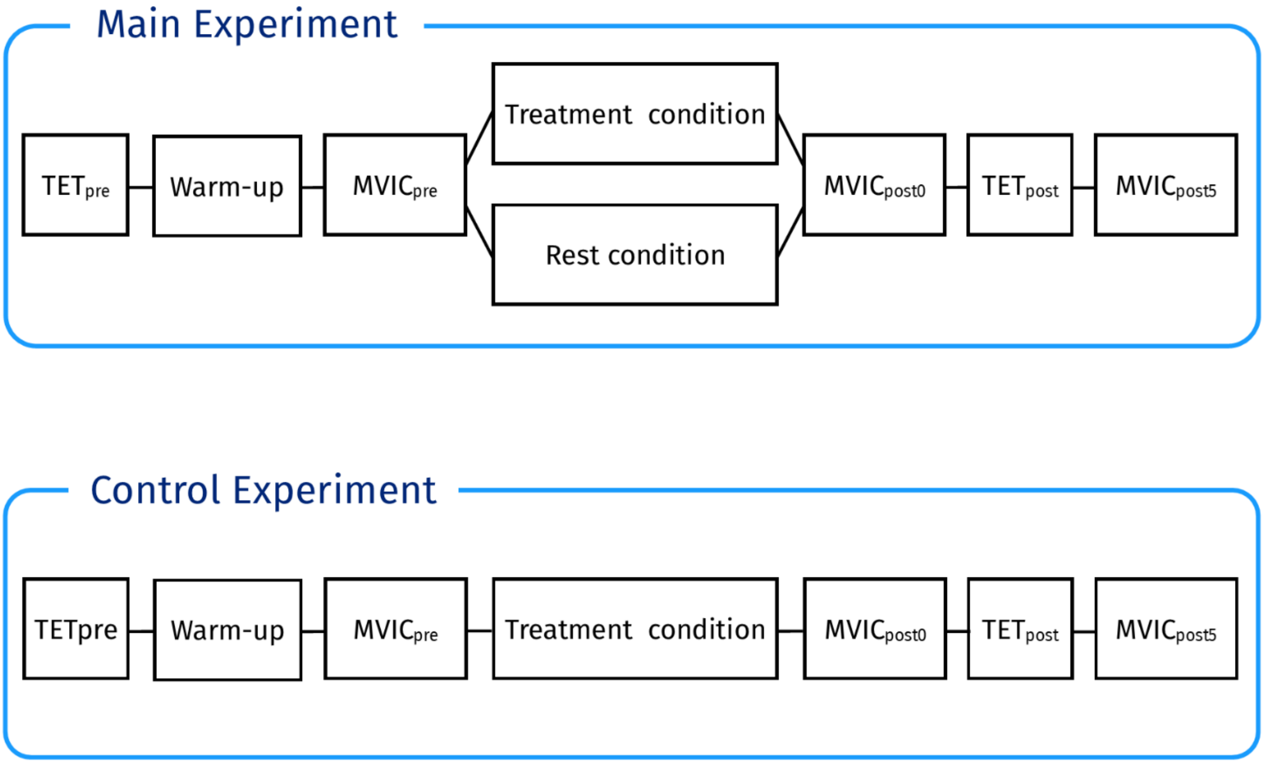

Figure 1.2.3.1 | study design in main and control experiments: In main experiment subjects come to the laboratory twice in which were administered a fatiguing task (Treatment condition) or a rest period (Rest condition). Subjects performed a temporal expectation task (TET) before (Pre) and after (Post) one of the two possible experimental conditions and a

maximal voluntary isometric contraction (MVIC) before (Pre), immediately after (Post_0) and five minutes after (Post_5) of it. In control experiment subjects performed exclusively the treatment condition.

Maximal Voluntary Isometric Contraction

Participants carry out a warmup in a cycle ergometer at low intensity for 5 minutes. Then they sat over an isokinetic dynamometer (System Pro 4, Biodex Medical System, New-York) with the right knee angle flexed at 90 degrees. In this position, they performed a series of submaximal contraction of the right quadriceps femoris.
Before starting the acquisition, participants performed two MVICs to familiarize themselves with the physical task, subsequently discarded. A resting time of 4 min was taken between each trial to avoid muscle fatigue on the measurements. Each MVIC lasted 4–5 s.

Experimental conditions

Treatment condition

Participants maintained as long as possible an isometric contraction of right quadriceps femoris set at 30% of the MVIC previously calculated, in the same position set during its assessment. Visual feedback was given to indicate the force applied by the leg. Participants had to increase the extension force to match a target line indicated on the visual display (corresponding on the 30% of MVIC) and maintain this force as steadily as possible. When the force fluctuation become consistent, an examiner strongly encourages the subject.
The time to exhaust (TTE) was calculated as the interval time elapsed from reaching the target force to the task failure, considered the instance in which the subject is unable to maintain the target force (30% of MVIC). The fatiguing protocol end at the SET8. Two minutes of rest were allowed between SETs.

Rest condition

Rest condition consists of 30 minutes of rest in which subjects were allowed to participate in social activity (e.g. speaking, chatting, etc.) but were asked to refrain from all high demanding cognitive activity (e.g. studying, etc.).

Temporal Expectation Task

The task applied in this study was a modified version of that designed by our group (4, 6) and exploited the temporal occlusion technique (18, 24). Participants were seated on a comfortable chair in a quiet room. They were requested to look at a computer screen where a video of a model running upon a track was displayed. The video (total duration about 5 s) showed, from the participant's perspective laterally to the track, a model standing and subsequently starting a run. The video ended when the model reached the arrival point, marked on the screen with a dot. The task consisted of observing the video that was partially occluded, from the onset of the trial, by a black window, that covered the last 1.5 second of the movement. During the occluded interval, subjects were required to indicate, by clicking on any button of the keyboard, when the movement reached its end, namely when they thought the model reached the dot marked on the screen. Once they pressed the key, the trial end. Participants did not receive any feedback on their performance throughout the whole session. They were just given the chance to watch the whole performance twice before starting the experiment (without obscuration, with the dot marked on the screen).

Participants performed 20 trials in both testing epochs, for a total of 40 trials per experimental condition. Instructions to the participants were written in a display window preceding the onset of the task; no further assistance was provided. The task has been programmed using dedicated software (E-Prime 2.0, SciencePlus) and took approximately 4 min. The experimental paradigm is summarized in the (Figure 1.2.3.2).

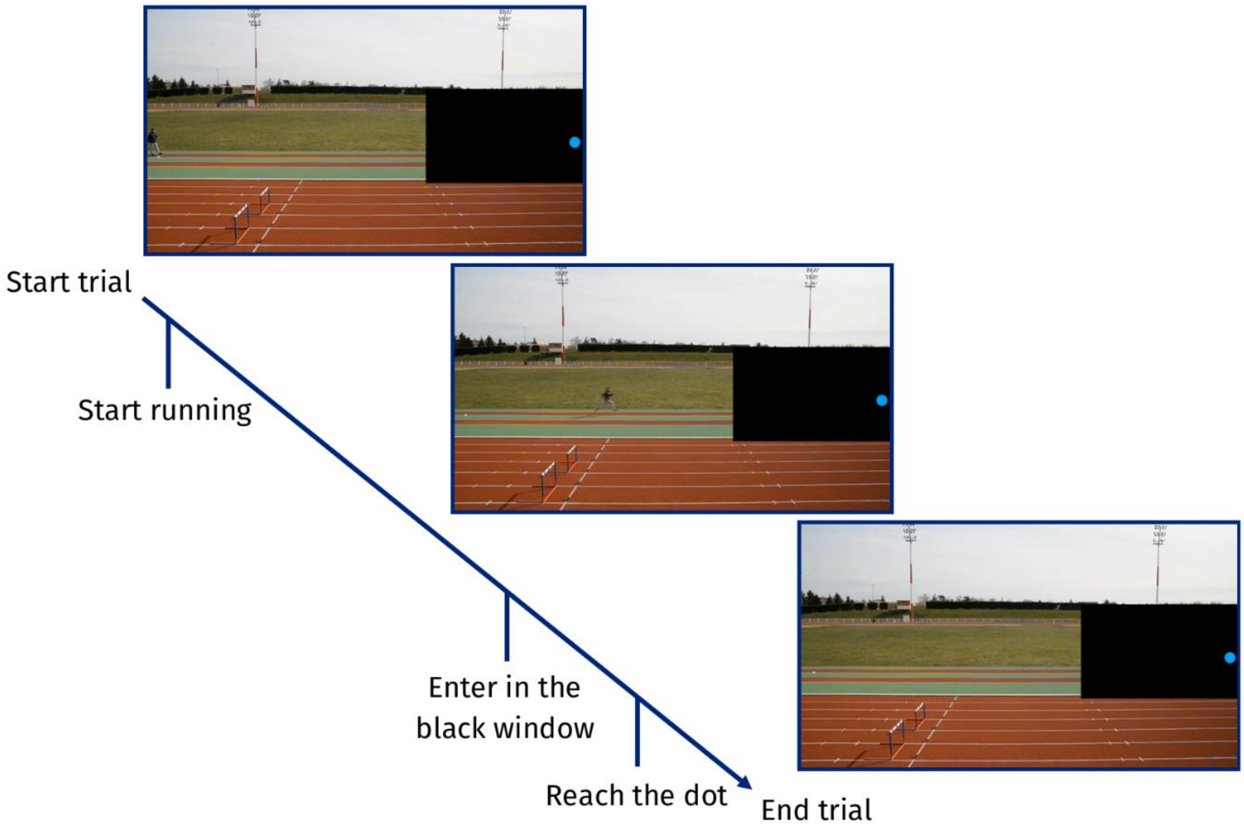

Figure 1.2.3.2 | Schematic view of the temporal expectation task. The video shows a model running upon a track. The dot, marked on the right of the display, indicates the arrival point of model's run. Subjects were required to indicate when they thought the model reached the dot.

Control Experiment

Subsequently, to assess the contribution of sensory-motor representation on the development of muscle fatigue, we tested 14 subjects utilizing the same treatment condition described above (see main experiment; experimental conditions; treatment condition; Figure 1.2.3.1) but using a different temporal expectation task. This consist of a marked dot that crosses the display from top to bottom. The dot followed a vertical tack and moving with a constant velocity. The video ended when the dot reached the arrival point, marked on the bottom of the screen. As in the previous temporal expectation task, this one consisted in the observation of partially occluded video, from the onset of the trial, by a window that covered the last 1.5 second of the dot movement. The duration of the movement was set equal to the duration of the previous temporal expectation task. Participants performed 20 trials for 40 trials and did not receive any feedback on their performance throughout the whole session. They were just given the chance to watch the whole dot movement twice before starting the experiment (without obscuration). The task was carried out using dedicated software (PEBL2) and modifying the open access conde of "Timewall. pbl". Task took approximately 4 minutes.

Data analysis

Physical measurement

Unpaired T-Test was performed to evaluate possible differences on MVIC between females and males. Subsequently, MVIC obtained at Post_0 and Post_5 were normalized in respect to the subjective MVIC obtained at Pre.

$$normalized\ MVIC = \frac{MVICpost}{MVICpre}$$

As the MVIC the TTE at SET8 was normalized in respect to the subjective TTE obtained at SET1.

$$notmalized\ TTE = \frac{TTEset8}{TTEset1}$$

Temporal expectation task measurement

Performance on the temporal expectation task was evaluated by means of response time, absolute values of timing error, percentage of anticipatory responses and coefficient of variation.

Response time (RT) is defined as the instance in which participants pressed a button of the keyboard, corresponding to the reproduced run duration replicated by subjects.

The timing error was calculated as the absolute difference between the RT and the real duration (D_{real}) of the video.

$$Absolute\ Timing\ Error = |RT - Dreal|$$

Percentage of anticipatory responses was calculated as the number of trials in witch subjects gave an anticipatory response (mainly when RT < D_{real}), normalized by the total of trials (20) and expressed in percentage.

Coefficient of variation was defined as:

$$Coefficient\ of\ Variation = \frac{SD}{mean}$$

Where SD and mean were referred to the difference between RT and D_{real} calculated for each trial.

Statistical analysis

All data are presented as means ± standard deviation of the means (SD).

Outlier values (more than twice the standard deviation) were removed from the analysis and the distributions of all variables were analysed with Shapiro-Wilk test.

Main Experiment

The effects of experimental CONDITION (treatment vs. rest) and TIME (Post_0 and Post_5) on normalized MVICs were tested using two-way fully repeated-measures ANOVA with TIME as within-subject factors (2 levels, Post_0, Post_5), and CONDITION as within-subject factors (2 levels, treatment vs. rest). Significant interactions between factors were examined with Post-Hoc Newman-Keuls comparisons.

Raw data of TTE during the eight SETs performed in the experimental treatment were analysed by means of a one-way ANOVA (factor TIME). Significant effect of factor TIME was examined with Post-Hoc Newman-Keuls comparisons.

RT were tested using two-way fully repeated-measures ANOVA with TIME as within-subject factors (2 levels, Pre, Post) and CONDITION as within -subject factors (2 levels, treatment vs rest).

Absolute timing error were tested using two-way fully repeated-measures ANOVA with TIME as within-subject factors (2 levels, Pre, Post) and CONDITION as within -subject factors (2 levels, treatment vs rest).

The effect of CONDITION and TIME on percentage of anticipatory responses and coefficient of variation, was analysed through non-parametric tests for dependent variable (Wilcoxon test).

To assess the relationship between temporal expectation ability and fatigue condition a multiple linear regression between absolute timing error (measured at Pre), normalized TTE and normalized MVIC was computed.

Control Experiment

The effects of TIME (Pre, Post_0 and Post_5) on MVICs were tested using one-way ANOVA with TIME as within-subject factors (3 levels, Pre, Post_0, Post_5). Significant effect of factor TIME was examined with Post-Hoc Newman-Keuls comparisons.

TTE during the eight SETs performed in the experimental treatment were analysed by means of a one-way ANOVA with TIME as within-subject factors (8 levels, from Set_1 to Set_8). Significant effect of factor TIME was examined with Post-Hoc Newman-Keuls comparisons.

RT were tested using a paired t-test (Pre versus Post treatment).

The effect of TIME on absolute timing error, percentage of anticipatory responses and coefficient of variation, was analysed through non-parametric tests for dependent variable (Wilcoxon test).

To assess the relationship between temporal expectation ability and fatigue condition a multiple linear regression between absolute timing error (measured at Pre), normalized TTE and normalized MVIC was computed.

In addition, we assess possible differences between treatment condition of main and rest experiment with a two-way ANOVA with TIME as within-subject factors (3 levels, Pre, Post_0, Post_5), and CONDITION as between-subject factors (2 levels, main experiment vs. rest experiment).

Results

Main Experiment

Physical measurement

All subjects were able to successfully complete the main experiment. One of them was rejected from the analysis because the unsuccess of treatment condition (his normalized TTE was greater than mean value + two times the SD). The raw values of MVIC were reported in Table 1.2.3.1.

Table 1.2.3.1 | Raw MVIC data obtained in the main and in the control experiments. Subjects performed the MVIC before (PRE), immediately after (POST0) and 5 minutes after (POST5) a fatiguing task (Treatment condition) or a rest period (Rest condition).

		PRE	POST0	POST5
MAIN EXPERIMENT	Treatment	238.89 ± 85.98 Nm	175.67 ± 76.27 Nm	182.44 ± 72.37 Nm
	Rest	230 ± 81.9 Nm	224.50 ± 76.60 Nm	225 ± 79.51 Nm
CONTROL EXPERIMENT	Treatment	270.64 ± 66.18 Nm	212.43 ± 52.94 Nm	212.14 ± 43.69 Nm

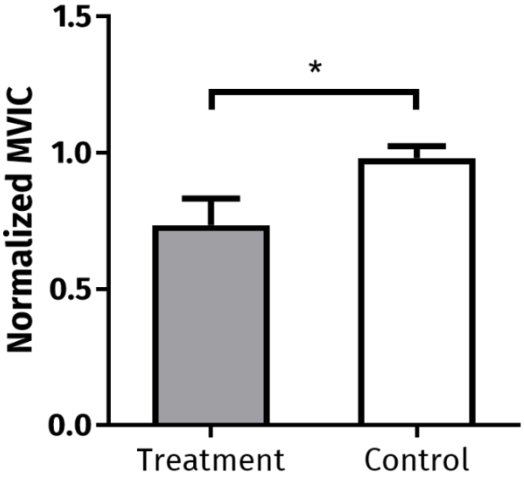

Figure 1.2.3.3 | Mean ± SE of normalized MVIC values obtained in the two experimental conditions. * Indicate significant effect of condition ($p < 0.001$).

The ANOVA on normalized MVIC values revealed a significant effect of CONDITION ($F = 157.91$; $p < 0.001$) (0.741 vs 0.978 for treatment and rest respectively) (Figure 1.2.3.3).

The mean values of TTE in the treatment condition were 120 ± 41 s, 80 ± 24 s, 71 ± 19 s, 65 ± 17 s, 65 ± 17 s, 64 ± 17 s, 58 ± 14 s, 60 ± 19 s from SET1 to SET8, respectively. The ANOVA

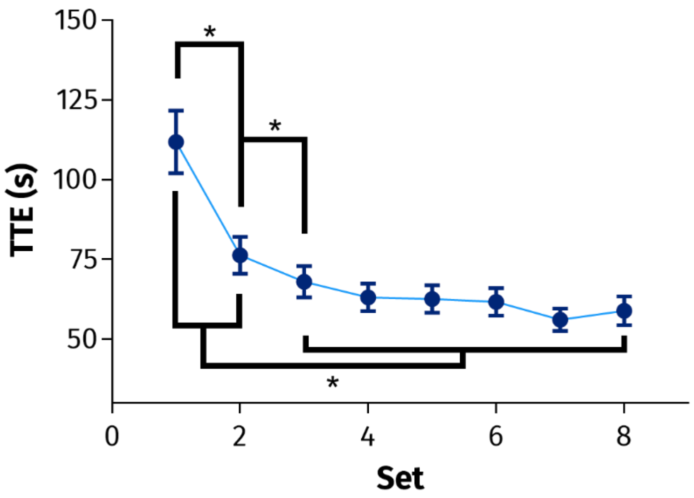

Figure 1.2.3.4 | Mean ± SE of subject's time to exhaust (TTE) in the eight SETs in the treatment condition of the main experiment. TTE significantly decrease from SET 1 to SET 3. The performance from SET 4 to SET 8 remain relatively stable. * Indicate significant effect of time (all p < 0.01).

showed a significant effect of TIME ($p < 0.001$). Post-Hoc analysis showed a significant decrease in TTE values between Set_1 vs. Set_2 and Set_3, and between Set_2 vs. Set_3 (p always < 0.01) (Figure 1.2.3.4).

Temporal expectation task measurement

The mean values of RT were 5563 ± 369 ms at Pre, and 5567 ± 421 ms at Post for the treatment condition, whilst they were 5576 ± 434 ms at Pre, and 5572 ± 462 ms at Post for the rest condition. No significant differences were observed on RT comparing the treatment with rest condition.

Absolute timing error were 305 ± 207 ms at Pre and 347 ± 233 ms at Post for the treatment condition and 403 ± 343 ms at Pre and 399 ± 380 ms at Post for the rest condition (see also Table 1.2.3.2). ANOVA did not show any significant effects. No other differences were found in other temporal expectation task measurement (coefficient of variation and percentage of anticipatory responses).

Table 1.2.3.2 | Absolute timing error obtained in the main and in the control experiments. Subjects performed the temporal expectation task before (PRE), and after (POST) a fatiguing task (Treatment condition) or a rest period (Rest condition).

		PRE	POST
MAIN EXPERIMENT	Treatment	305 ± 207 ms	347 ± 233 ms
	Rest	403 ± 343 ms	399 ± 380 ms
CONTROL EXPERIMENT	Treatment	292 ± 82 ms	331 ± 158 ms

A significant positive correlation was found between the absolute timing error Pre and normalized TTE ($p = 0.003$; $r^2 = 0.402$) (Figure 1.2.3.5).

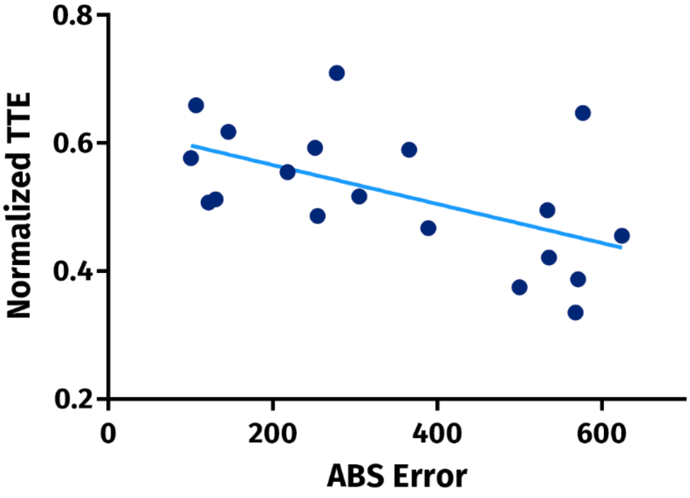

Figure 1.2.3.5 | Correlation between absolute timing error measured at PRE (ABS Error) and the decrease in time to exhaust (Normalized TTE).

Control Experiment

Physical measurement

All subjects were able to successfully complete the control experiment. The mean values of raw data of MVIC were reported in Table 1.2.3.1. The ANOVA on normalized MVIC values revealed a significant effect of TIME ($p < 0.001$). Post-Hoc analysis showed a significant decrease in MVIC values after the treatment condition (as reported in the main experiment).

The mean values of TTE were 83.07 ± 21.09 s, 69.21 ± 25.25 s, 60.57 ± 16.13 s, 58.07 ± 17.96 s, 55.50 ± 16.56 s, 35.93 ± 17.18 s, 51.21 ± 15.74 s, 53.93 ± 19.51 s from Set_1 to Set_8, respectively. The ANOVA on TTE values showed a significant effect of TIME ($p < 0.001$). Post-Hoc analysis showed a significant decrease in TTE values between Set_1 vs. Set_2 and Set_3, and between Set_2 vs. Set_3 (p always < 0.01).

Temporal expectation task measurement

The mean values of RT were 5418 ± 279 ms at Pre, and 5471 ± 367 ms at Post for the treatment condition. No significant differences were observed on RT comparing the values obtained at Pre and at Post.

Absolute timing error were 292 ± 82 ms at Pre and 331 ± 158 ms at Post (see also Table 1.2.3.2). Paired T-Test did not show a significant difference between Pre and Post values as in the main experiment. No other differences were found in other temporal expectation task measurement (coefficient of variation and percentage of anticipatory responses).

No significant correlation was found between the absolute timing error Pre and normalized TTE in the control experiment.

Discussion

The present study focused on possible alteration on temporal expectation ability associated to a motor fatigue condition. We induced a fatigue condition in the right quadriceps femoris through a series of submaximal isometric contraction maintained as long as possible. Our protocol induced a decrease in maximal voluntary isometric contractions but did not alter the ability of temporal expectation. This last one, seems to be a predictor of subjective resistance to isometric contractions only when is measured through a specific temporal expectation task (i.e., running model vs moving dot) in which a real movement was observed.

Comparing the MVICs obtained in treatment condition whit those obtained in rest condition emerges the effectiveness of our protocol to induce a fatigue condition. Data highlight a decrease in MVIC in participants only after the treatment condition. Furthermore, the reduction in MVIC was maintained also after the execution of the temporal expectation task. This suggest that the subject’s condition of fatigue does not change during the application of the cognitive task and that the recovery allowed does not alter this condition, which persist for more than 5 minutes.

Our results indicate also that a muscular fatigue condition does not alter the temporal expectation ability, this assumption is supported by no differences found in the factor TIME and in the factor CONDITION of the ANOVA performed whit all cognitive task measurements in both experiments.

This could appear in contrast whit results obtained by Demougeot and Papaxanthis (7), who spotlighted the strong influence of muscle fatigue on sensory-motor representation, this last one essential to goodly execute a temporal expectation task (6).

A previous study utilising temporal occlusion technic (6) just speculated the possibility that during the occlusion interval participants mentally simulate the previously observed action. Following this assumption, we expected that the altered subjective sensory-motor representation will induce an increase in absolute timing error measured after muscle fatigue.

The lack of differences found in our experiment could be explained considering the presence in our protocol of visual feedback, which could compensate the altered sensory-motor representation, allowing subjects to correct the given response to the cognitive task despite their altered state.

A fMRI study (17) identified a strong overlap between the neural activations reported during motor imagery and observation of whole-body movements. The brain region interested can be found in classic motor areas like primary motor cortex (M1) and supplementary motor area (SMA), but also in the cerebellum and sections of the basal ganglia.

Since Eaves and colleagues (9) demonstrated that motor imagery can be performed also during action observation, we could suppose that in a temporal expectation task like that used in our study, the motor imagery processes start with the observation of the action shown in the task and are maintained also when the observed action is occluded.

If we accept this assumption, it will mean that the process of motor imagery starts before the occluded interval and that it could be influenced by the action observation performed at the beginning of the task. This last one, could compensate the altered sensory-motor representation and positively influence the motor imagery process, resulting in no observed differences in all cognitive task measurements.

To better understand the validity of this hypothesis, future studies will investigate the influence of an increasing in the time of obscured movement and a decreasing in the time of observed one (e.g. whit a larger black window).

In our study, we found a positive significant correlation between cognitive and motor performance. Specifically, we observed that people with more accurate temporal expectation ability are those who maintain motor performance more constant, presenting a minor decrease in TTE and MVIC. Interestingly, these results emerge only when a specific temporal expectation task was utilized, in which a real movement was observed (main experiment).

Previous studies suggested that the ability of temporal expectation depend on subjective sensory-motor representation (5, 6, 13, 27). The motor representation results strictly related to the physical practice. Indeed, the constant use of specific body part induces a reorganization and an enlargement of motor cortex representation (29) while the disuse or the immobilization of limbs movements significantly reduce the cortical representation of muscles immobilized (14). The enlargement of motor representation was associated to learning-dependent dendritic spine growth observed in both superficial (21) and deep (20, 33) layer motor cortex neurons, including in corticospinal neurons (31).

At the peripheral level, Adam and colleagues (2) observed that a specific preferential use could cause changes in motor recruitment strategies. In fact, the motor units of the dominant hand present a lower mean value for recruitment threshold, initial firing rate, average firing rate, and discharge variability when compared with the motor unit of nondominant hand These changes in motor recruitment strategies seems a result to adaptations in response to preferential use rather than specific training of endurance performance. Similar results were obtained after 6 weeks of endurance training program (28), which cause a significant decrease in the motor unit discharge rate after endurance training.

Interestingly, both studies (2, 28) reported an increase in motor control (highlighted by a decrease in force fluctuation) in muscle that present a motor unit discharge decreased when an isometric contraction was performed (30% of MVC).

This supports our hypothesis in which better motor recruitment strategies could positively impact on temporal expectation and endurance ability, resulting in a strong correlation between those two types of performances.

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1.2.4 Action observation training and motor fatigability

Introduction

Action observation (the process of observing actions performed by others) is a mental technique able to activate motor areas partially overlapped with those activated by movement execution (14, 18, 33). This unique feature was attributed to the presence of a mirror neuron system: special groups of neurons located in the prefrontal cortex which map the visual representation of the observed action onto our motor representation of the same action (16, 31).

Researchers tried to adopt action observation (AO) as new motor learning techniques obtaining results fairly encouraging (1, 5, 7, 13, 36). Indeed, AO training (consisting of the repetitive observation of specific movement) seems to enhance the motor performance output (behavioural level) (11, 22) and to produce brain modification associated with neuroplasticity (neurophysiological level) (20, 38), two adjustments necessary to obtain motor learning (37).

Though the continued observation and the execution of distinct movements boost motor performance, practice it for an extended period could produce an adverse effect. People who sustain a cognitive or physical activity for a prolonged time may experience a feeling of tiredness or even exhaustion denoting fatigability (28, 34). This condition negatively affects motor and cognitive performances. For example, motor fatigue can result in motor performance deterioration and depression of motor evoked potentials (MEP) induced by transcranial magnetic stimulation (TMS) in the muscles involved in the exercise. This has been demonstrated to occur either after the execution of a maximal voluntary contraction continued until the exhaustion (12) and after the execution of a tapping task at the maximal frequency for about 1 min (35, 41). In both cases, the decrease of cortical excitability, termed post-exercise depression, has been attributed to the onset of "central fatigue" (12, 23, 40). Avanzino and colleagues (2) observed that the execution of finger opposition movements' sequence lasting 5 min induced a motor performance deterioration, a decrease in cortical excitability but no change in maximal voluntary contraction. In addition, after 10 min of rest, the motor performance recovers, but the cortical excitability remained depressed, highlighting that the recovery of motor performance does not follow cortical excitability dynamics.

As a motor practice, also mental activity could induce a detrimental state inducing declines in several executive functions (8, 17, 19, 25–28, 30).

Lim and colleagues (24) showed that after a 20-min continuous psychomotor vigilance test, subjects displayed progressively slower reaction times and a significant increase in mental fatigue ratings after the task.

Similarly, motor imagery (the mental simulation of action without its actual execution) (18), a mental technique quite similar to AO, induce fatigue if performed for a prolonged time.

In Talukdar's study (39), participants were asked to perform the motor imagery tasks for one session lasting more than one hour. The authors collected pre- post-test self-report fatigue measures and EEG data, which showed a motor imagery-induced mental fatigue.

Despite studies described above provided specific influence of motor and mental fatigue in the related domain, the possible effect that a prolonged mental activity could induce in motor outcomes still remain little explored. Since AO is a cognitive activity that activates several common areas shared with action execution, it is the best object of study to investigate possible effects prompted by its excessive activity.

This study aimed to verify if the changes observed in cortical excitability and motor performances dynamics following the execution of finger opposition movements' sequence are present also after AO training. Results will help research to program proper AO training to minimize the negative effect due to this activity.

Materials and methods

Participants

Thirty-seven healthy young adults (mean age 23.5 ± 4.1 years, 19 males and 18 females), recruited from the University of Genoa, volunteered to participate in this study. Twenty of them were enrolled in experiment 1 and the other seventeen were enrolled in experiment 2. They were all right-handed according to a modified Italian-translated Edinburgh Handedness Inventory.

All participants had no contraindication to TMS or any history of neurological or psychological disorders. Informed consent was obtained according to the policy of our Institution and to the Declaration of Helsinki.

Experimental procedure

Participants enrolled in experiment 1 joined the laboratory two times. On both occasions, participants performed one of two possible conditioning protocol (see paragraph below) in a random and counterbalanced order. In each session, lasting about 90 minutes, behavioural and neurophysiological measurements were collected.

Participants recruited in experiment 2 attended the laboratory once and performed a single conditioning protocol that combines action observation (AO) and electrical stimulation (ES) techniques (see paragraph below). As in experiment 1, the session lasts about 90 minutes, and subjects were evaluated at the behavioural and neurophysiological level.

All participants were evaluated before (Pre) the conditioning protocols, immediately after (Post_0) and 60 minutes after them (Post_60) (Figure 1.2.4.1).

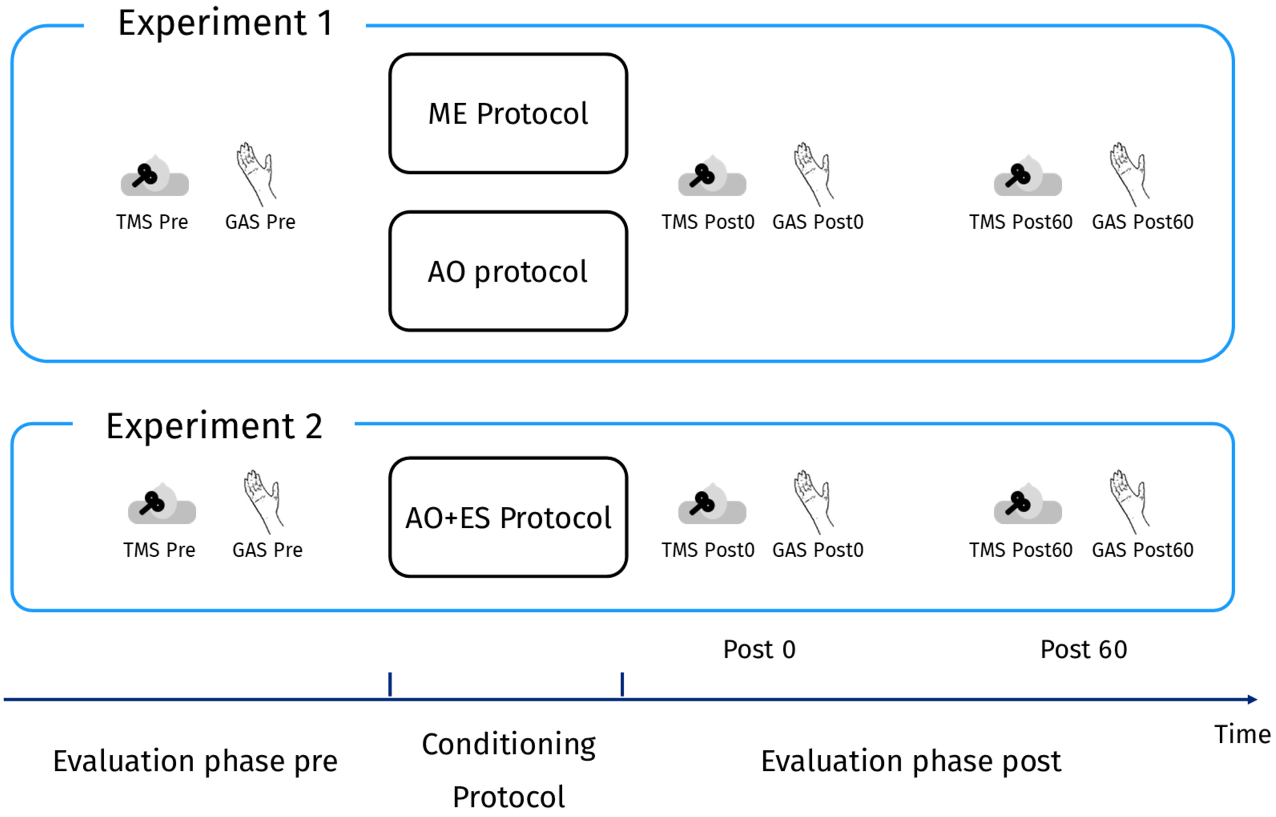

Figure 1.2.4.1 | Experimental procedure. In experiment 1 participants joined the laboratory twice and performed one of two possible conditioning protocol in a random and counterbalanced order. In each session, lasting about 90 minutes. In experiment 2 participants recruited in experiment 2 attended the laboratory once and performed a single conditioning protocol that combines action observation (AO) and electrical stimulation (ES) techniques. In both experiments behavioural (GAS) and neurophysiological (TMS) measurements were collected.

Neurophysiological measurements

Neurophysiological measurements were obtained using transcranial magnetic stimulation (TMS). TMS was performed with a Magstim 200 stimulator (Magstim Company, Dyfed, UK). During TMS, the subjects maintained contralateral muscle at rest. A figure-of-eight shaped coil (external diameter: 9.5 cm) was placed over the optimal position of the scalp for evoking MEPs in the contralateral APB muscle. After that, the stimulus intensity producing MEP amplitudes of about one mV at rest was established. The mean amplitude (peak-to-peak of the EMG signal) of the responses was calculated. In experiment 1, a total of 180 stimulations were collected for each subject: 30 for each epoch (Pre; Post_0; and Post_60) in both days.

In experiment 2, a total of 90 stimulations were collected for each subject: 30 for each epoch (Pre; Post_0; and Post_60).

Behavioural measurements

Participants wore a sensor-engineered glove (Glove Analyzer System, GAS; ETT S.p.A., Italy) on their right hand. The glove, made in lycra, had on the top of each finger conductive wires placed to record the contact between the thumb and the other fingers. This system was previously used to study finger motor performance in healthy subjects (11) and neurological patients (9, 32). In the present study, this system allowed the evaluation of the kinematic properties of finger's movement, and spatial accuracy.

Participants execute the following motor sequence: opposition of thumb to index, medium, ring and little fingers (SEQ). They practised the task at their own pace, and the training generally ended within 1 min, when they were able to execute the task without errors. Then, subjects perform SEQ following an acoustic cue paced at 2 Hz for 1 min.

Data from the glove were processed with customized software (GAS, ETT, S.p.A., Italy). Touch duration (TD; i.e., the contact time between the thumb and another finger, ms), inter-tapping interval (ITI; i.e., the time interval between the end of a thumb-to-finger contact and the beginning of the following contact in the finger motor sequence, ms), and the percentage of correct sequence on the total performed (%CORR) were extracted from the acquired data.

Conditioning protocol

Experiment 1

In the experiment 1 subjects performed two conditioning protocol: one, named ME protocol (motor execution), consisted in repeating a specific motor task with the hand's fingers, and the other, called AO protocol (action observation), consisted in observing a video representing the same motor task of ME protocol.

ME protocol

During ME protocol, subjects executed the same motor sequence used in the evaluation phase to assess behavioural data (SEQ). Participants perform SEQ following an acoustic cue paced at 2 Hz for 1 min. When the trial ended, they rested for 10 seconds and then repeated the trial described above. The ME protocol ended at the eighth trial.

AO protocol

During AO protocol, subjects observe a video recorded ad-hoc showing a right hand performing repetitive SEQ. This movie clip was obtained by filming on a black background the right hand of a human demonstrator who performed SEQ paced at 2 Hz. The video last 1 min and the thumb fingers touch was synchronized with an acoustic cue paced at 2 Hz. When the video ended, subjects rested for 10 seconds and then restarted to observe the clip. The AO protocol ended at the eighth trial.

Experiment 2

In experiment 2, subjects performed one conditioning protocol combining action observation (AO) and peripheral electrical nerve stimulation (ES) techniques (see paragraph below). The conditioning protocol, named AO + ES protocol (Action observation + electrical stimulation), consisted of observing a video representing SEQ contemporary to electrical stimulation.

AO + ES Protocol

During AO + ES protocol, subjects experienced the AO protocol described above associated with electrical stimulation. The frequency of the electrical stimulation was set to administer to the subject an electrical stimulus to every finger’s opposition movement (in correspondence to the thumb-finger closing phase), similar to that just already proposed in our previous study (4). A MatLab custom-made software managed the synchronization between the video presentation and the electrical stimulations. Electrical stimuli were applied through a bipolar electrode (cathode proximal) connected to a Digitimer constant current stimulator (DS7AH HV, Digitimer Ltd., UK), using square wave pulses (duration 1 ms) at an intensity of three times the perceptual threshold. To find the perceptual threshold, the experimenter placed the electrode on the right wrist to correspond to the median nerve location. The electrical stimulation was delivered at different intensities in a random order to find the lowest intensity perceived by the participant, whom the experimenter verbally questioned. This value was considered the perceptual threshold. Then, the intensity of stimulation was increased to three times the perceptual threshold, intensity able to excite the motor fibres of the mixed median nerve, i.e., to evoke a slight twitch in the innervated muscle (abductor pollicis brevis). All subjects tolerated this intensity of stimulation.

Data Analysis

Data distribution was assessed using the Shapiro-Wilk test. MEP amplitude, TD and ITI values were normally distributed, whereas %CORR were not.

For experiment 1, normally distributed data were analysed by means of two-way fully repeated-measures ANOVA with EPOCH as within-subject factors (3 levels: PRE, POST 0, POST 60), and PROTOCOL as within-subject factors (2 levels: ME vs AO). Bonferroni comparisons examined significant interactions between factors. Friedman test was used to compared %CORR within group, whilst Wilcoxon tests were applied to evaluate difference between groups in the three testing epochs.

For experiment 2, we compared behavioural and neurophysiological measurement obtained with those obtained in experiment 1. To do that, we used two two-way ANOVA, one with EPOCH as within-subject factors (3 levels: PRE, POST 0, POST 60), and PROTOCOL as between-subject factors (2 levels: ME vs AO+ES), and the other with EPOCH as within-subject factors (3 levels: PRE, POST 0, POST 60), and PROTOCOL as between-subject factors (2 levels: AO vs AO+ES). Post-Hoc Bonferroni comparisons examined significant interactions between factors.

Results

Experiment 1

The results of the ANOVA on MEP amplitude values (Figure 1.2.4.2) showed significant main effects of PROTOCOL ($F_{(1,19)}$ = 4.97, p = 0.038) and TIME ($F_{(2,38)}$ = 3.46, p = 0.042), as well as a significant TIME*PROTOCOL interaction ($F_{(2,38)}$ = 3.82, p = 0.031). Bonferroni post hoc indicated that MEP amplitude decreased significantly immediately after ME (POST 0 vs. PRE: p = 0.012), whilst no differences before and after AO protocol administration. Furthermore, the comparison between groups showed that at PRE MEP amplitude in ME and AO were comparable, but they were significantly lower in ME at both POST 0 (p = 0.047) and POST 60 (p = 0.031).

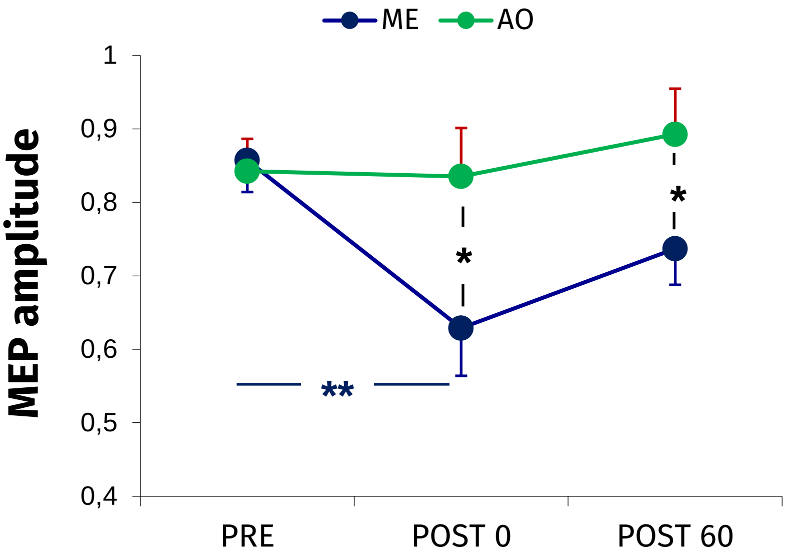

Figure 1.2.4.2 | Mean ± SE of neurophysiological measurement. MEP amplitude recorded by transcranial magnetic stimulation (TMS) before (PRE) immediately after (POST 0) or 60 minutes after (POST 60) the ME (Blue) and AO (Green) protocols.

The statistical analysis on TD and ITI showed no significant effect of EPOCH, PROTOCOL, and no significant interaction. Friedman test on %CORR found a significant effect of EPOCH only in ME ($\chi^2(2,20) = 6.54$, $p = 0.038$). Post hoc analysis revealed that %CORR in POST0 decreased significantly with respect to PRE ($p = 0.014$). No differences within epochs in AO and no differences between groups were found. Results are offered in Figure 1.2.4.3.

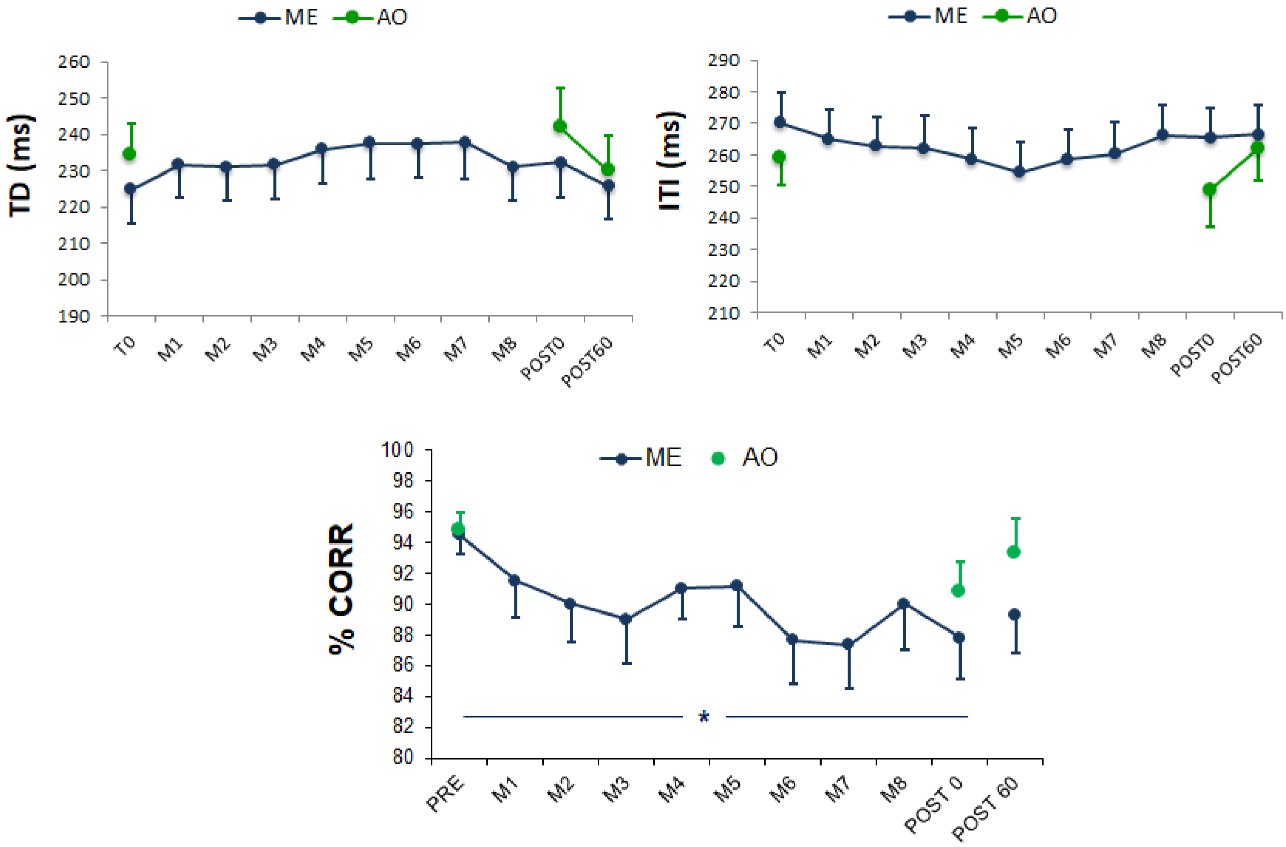

Figure 1.2.4.3 | Mean ± SE of behavioural measurement. Collected with the Glove Analyzer System, (GAS) before (PRE) immediately after (POST 0) or 60 minutes after (POST 60) the ME (Blue) and AO (Green) protocols. Touch duration (TD; and inter-tapping interval (ITI) do not show any statistical differences, while the percentage of correct sequence on the total performed (%CORR) suffers a progressive decline during the ME protocol (Effect of TIME).

Experiment 2

The results of the ANOVA on MEP amplitude values (Figure 1.2.4.4) showed significant effect of TIME ($F_{(2,32)} = 4.086$; $p = 0.026$), Newman-keuls comparison indicated that MEP amplitude decreased significantly immediately after (POST 0 vs. PRE: $p = 0.021$) and 60 minute after AO + ES (POST 60 vs. PRE: $p = 0.044$).

The statistical analysis on TD, ITI and %CORR showed no significant effect of EPOCH.

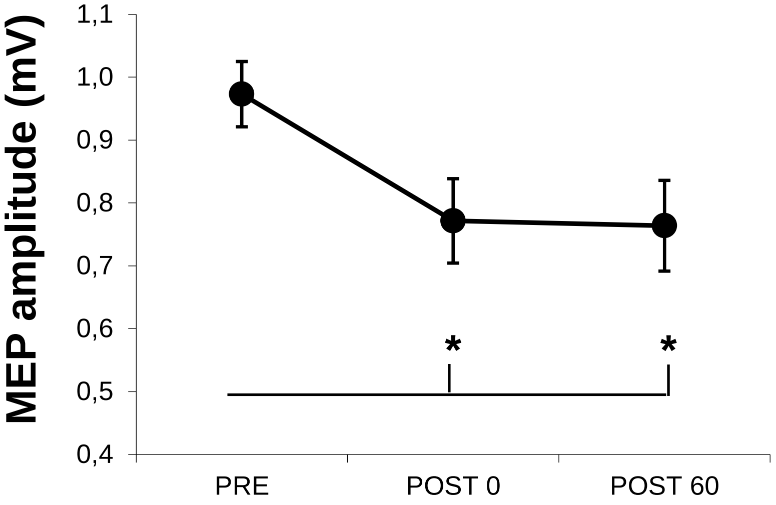

Figure 1.2.4.4 | Mean ± SE of neurophysiological measurement. MEP amplitude recorded by transcranial magnetic stimulation (TMS) before (PRE) immediately after (POST 0) or 60 minutes after (POST 60) the AO + ES protocol.

Discussion

This study aimed to verify possible changes following an AO training of a finger opposition movements' sequence. Two experiment collecting behavioural and neurophysiological measurements were conducted. In the first, subjects were exposed to two conditioning protocols consisting in a sequence of repetitive movements (ME protocol) of the APB or an observation (AO protocol) of the same movement for the same time. In the second, participants were exposed to one conditioning protocol which combine the action observation of the APB finger opposition movements with the electrical stimulation able to excite the motor fibres (AO+ES protocol).

During ME protocol was registered a significant decrease in both neurophysiological and behavioural measurements. Cortical excitability significantly decreased after the ME, showing MEP amplitudes smaller in POST 0 than in PRE. This decrease in cortical excitability seemed partially recovered after 60 min of rest.

Similarly, also motor performance decreased following the same pattern of the neurophysiological measures. The percentage of correct response (% CORR) suffered a progressive decline which resulted in a decrease in performance immediately after the end of ME protocol which recovered after 60 min of rest. These modifications are in line with previous studies showing that the execution of finger opposition movements' sequence induced a motor performance deterioration and a decrease in cortical excitability (2).

Different results were obtained after AO protocol. The mere observation of the finger opposition movements' sequence did alter neither the cortical excitability nor all behavioural measurements. This trend was highlighted by the differences observed in neurophysiological measures between protocols in POST 0 and POST 60. Indeed, cortical excitability was higher in AO rather than in ME protocol in both epochs.

The ability of AO training to enhance the motor performance (11, 22) and to produce neuroplasticity (38) without induce a fatiguing condition, makes it a suggested tool to schedule neurorehabilitative interventions, especially in patients how report fatigue as common symptom (e.g., in multiple sclerosis or Parkinson) (15, 21).

Experiment 2 showed a partial confirm of the results obtained during ME protocol. When AO was associated with electrical stimulation, AO + ES produced similar decrease in cortical excitability as those reported during ME, but no changes in behavioural measurements. This phenomenon could be produced by the afferent signals that modulate the level of corticospinal excitability. Bisio et al. (3) observed that to evoke plasticity in the human motor system, AO must be combined with afferent inputs from periphery. Similarly, also other afferent input such as muscle vibration (i.e. a proprioceptive stimulation, able to evoke a kinaesthetic illusion of movement) can modulate the corticospinal excitability up to 60 minutes if associated with action observation (6). Further, to evoke behavioural improvements AO needs to be combined with ES (4). Based on these studies, we can speculate that the presence of afferent signals could be necessary cause a cortical plasticity, performance improvements, and similarly to what happens during prolonged motor execution, fatigability expressed as post-exercise depression, but are not sufficient to produce a behavioural outcome. We must point out that in this study we do not collected a self-reported measures of fatigue (such as the rate of perceived exertion (10) which were showed to be most related with motor performance (29).

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CHAPTER 2

2.1 Activity of the corticomotor system as a function of muscle length and type of contraction

The corticospinal system is the central motor system for controlling movements that require the most excellent skill and flexibility (22). It connects the frontal and anterior parietal lobes with the spinal grey matter. The first is the motor system's primary region and works in association with other motor areas, including the premotor cortex, supplementary motor area, posterior parietal cortex, and several subcortical brain regions (15, 22).

The premotor cortex and supplementary motor area have specific roles in the temporal control (3, 16), coordination of movements (28), motor learning (17) and in movement preparation (3) and executions (14).

The posterior parietal cortex has been implicated in sensorimotor integration (3, 6), in visually guided movements (5, 6) in the transformation of sensory data into coordinate frameworks appropriate for movement generation, and in selection of movements for execution (3, 8).

Neurophysiological studies have suggested that these brain regions work simultaneously during the planning and execution of movements (4, 12, 27) rather than sequentially. These findings challenge the classical serial model of brain processing: a response is produced step-by-step, transferring the information from one region to another, and suggesting that the corticospinal system processes sensory information in parallel (23).

Evidence of this cooperative work between motor and sensory brain regions are reported from studies investigating the activity of the corticomotor system in relation to action observation, nociceptive stimulation (26), muscle vibration (31), muscle length, and limb position (11, 24).

A huge number of studies previously demonstrated that special visual stimuli are able to modulate the primary motor cortex and to induce cerebral plasticity (for details see Chapter 2, paragraph: Motor imagery and action observation).

Studies highlighted also that an application of a short-lasting nociceptive stimulus prior to a TMS pulse (with an interstimulus interval of + 50 ms of latency) elicits a decrease in MEPs amplitude (2, 32).

Muscle vibration, a series of proprioceptive stimuli that evokes the illusion of limb’s movement, generate an increase in cortical excitability. Smith (31) submitted to subjects’ a proprioceptive stimulation protocol consisting in a 30s on/15s off duty cycle of muscle vibration for 15 min or 30 min. An increased in MEP amplitude and enlarged area of motor cortex representing of stimulated muscle was detected after 15 min of vibration, but no changes were associated after 30 min of it.

Using a comparison between TMS and transcranial electric stimulation, Kossev (18) demonstrated that the facilitation of MEPs recorded from the vibrated muscle was of intracortical origin. In the electroencephalographic (EEG) study Munte and colleagues (25) reported additional evidence for a motor cortical activation during muscle vibration. This cortical modulation induced by proprioceptive stimuli was associated to movement illusion generated by muscle vibration (13, 21).

In upper limbs, the influence of proprioceptive system in cortical excitability was observed also after no-external stimulations. Ginnaneschi and colleagues (11) examined whether the recruitment properties of the corticospinal pathway to intrinsic hand muscles are influenced by variations of the shoulder joint angle. Results obtained showed that shoulder position influences the recruitment efficiency (gain) of the corticospinal volleys to motoneurons of intrinsic hand muscles. It is proposed that activity of peripheral receptors signalling static shoulder position influences corticomotor excitability of hand muscles both at the cortical and at the spinal level.

Similarly, Mogk et al. (24) observed changes in corticospinal excitability of posterior deltoid and biceps brachii associated with multi-joint changes in static upper limb posture.

Muscle length seems responsible also for changes regarding spinal activation. M-wave amplitude is sensible to modification of limb position which modulates muscle length (20), suggesting that the impaired MVC in shorter muscle length would partially result from an alteration of the neuromuscular propagation.

When considering supraspinal factors, the contribution of muscle length seems less effective: no results were obtained in the upper limb (10), while in the lower limb, the effect of muscle length is significant only when studied in a more “active” context (during a movement execution or a static contractions) (7).

Corticospinal excitability in lengthening and shortening contractions was firstly investigated by Abbruzzese (1) in elbow flexor muscles. Abbruzzese found that brain stimulation evoked larger motor potentials during performance of a voluntary contraction requiring muscle shortening than muscle lengthening. Similar results were most recently observed by Sekiguchi et al. (29). In this work they applied TMS over primary motor cortex (M1), corresponding to elbow flexor muscles, using different stimulus intensities. Furthermore, they included the analysis of input-output property in the corticospinal tract, that shows a typical sigmoidal shape. Authors found that both the plateau value, consisting of maximal MEP value reached during the different stimulus intensities, and maximum slope, consisting on maximal increasing in MEP values from two stimulus intensities, were significantly lower during lengthening rather than shortening contraction.

Opposite findings were observed during lengthening and shortening contractions of first dorsal interosseus muscle (FDI) (30) which present a maximum slope and threshold, costing on the lowest stimulation intensity that elicited at least half MEP, significantly higher during eccentric than during concentric contractions, maintaining the same levels of plateau value.

These findings highlight different motor recruitment strategies between contractions.

A Fang's study (9) suggested that eccentric movements needed a significantly longer time for early preparation and a significantly greater magnitude of cortical activity for later movement execution. The extra preparation time and higher amplitude of activation may reflect CNS activities that account for the higher risk of injury, higher degree of movement difficulty, and unique motor unit activation pattern associated with maximal-level eccentric muscle actions.

Accordingly, a fMRI study conducted by Kwon et al. (19), showed that lengthening and shortening muscle contractions induced different patterns of cortical activity. Their findings revealed that cortical areas associated with motor performance were mainly activated in eccentric contractions, including pre-supplementary motor area, prefrontal cortex and anterior cingulate cortex, areas known to be involved in the attention ability (33).

This paragraph presents two studies investigating the function of muscle length and type of contraction on the corticomotor system.

The first study examined the influence of hip and knee angle on the neuromuscular function, architecture, and corticospinal excitability of knee extensors.

The second study investigated the difference in primary motor cortex (M1) excitability when preparing concentric and eccentric contractions.

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2.2 Experimental Contribution

2.2.1 Effect of knee and hip angles on knee extensor torque: neural, architectural, and mechanical considerations

Introduction

Gordon, Huxley, and Julian's pioneering works demonstrated the parabolic shape length-tension relationship of isolated muscle fibers, and the effect muscle length exerts on force production capacity (14). In-vivo, this relationship transcribes for instance, by the lower maximal voluntary isometric contraction (MVIC) torques developed at a moderate flexed knee position (e.g., 35° knee flexion, 0° = full extension) compared to more flexed positions (55° and 75° knee flexion) (1). Babault and colleagues also reported lower twitch torque amplitude elicited on the knee extensors by percutaneous nerve stimulation in a moderate flexed position (35° knee flexion) compared to more flexed positions (55° and 75°) (1). A similar change between MVIC and evoked twitch torques as a function of knee joint angles conducted these authors to suggest that lower MVIC would be explained by a reduced number of attached cross-bridges related to short muscle length (1). The knee extensors encompass the three mono-articular vastii muscles (vastus lateralis, vastus intermedius, vastus medialis) and the bi-articular rectus femoris (RF) muscle. Therefore, the hip joint position could influence the knee joint's torque due to the bi-articular RF muscle's contribution (17). The greater knee extensors MVIC torque developed in a seated compared to a supine position (i.e., flexed vs. extended hip position) support this assumption (9). However, recent findings reported no difference in knee extensors MVIC torque performed in a seated or supine position (5). Furthermore, while a reduction in the evoked twitch torque amplitude of the knee extensors occurred in a seated compared to a supine position with a 90° knee flexion (21), no difference was observed between these two positions for lower knee flexion angles (20° or 60°) (5). Concomitantly to greater MVIC torque, Cavalcante and colleagues measured lower pennation angle and longer fascicle length for the VL and RF muscle at 60° knee flexion than 20° knee irrespective of hip position (5). The greater stiffness of the RF muscle than the vastii muscles, when lengthened beyond the slack angle (around 54° of knee flexion for a 10° hip extension) (28), could partly account for these discrepancies between studies. Hip angulation could thus modulate the position75 torque relationship of the MVIC of knee extensors. However, the paucity of muscle length measurement made it challenging to determine the hip position's effect on knee extensors' muscle length.

In addition to changes in contractile properties, Maffiuletti and Lepers showed that the neural drive sent to target muscles was altered in the supine position (21). Similarly, reduction in voluntary activation and MVIC was reported for the knee extensors when increasing knee extension (8, 20). However, the role of central factors remains discussed since no change in voluntary activation has been reported by other authors (1, 3). Recruitment of different synergist muscles can vary according to the joint position. Rochette and colleagues found, for instance, greater activity of the RF muscle than the vastii muscles during submaximal voluntary contractions (20% MVIC), performed in a supine or a seated position with a 90° knee flexion (24). Such difference could reflect optimization of the voluntary drive between the knee extensors' synergist muscles (18). Hence, the use of the twitch interpolated technique or the EMG analysis of a single muscle of the knee extensors remains limited to infer changes in the voluntary drive during voluntary contraction between synergist muscles. The analysis of the EMG activity of different synergist muscles of the knee extensors with different hip or knee angulations is required. In this attempt, single-pulse transcranial magnetic stimulation could represent an alternative technique to quantify the corticospinal pathway's excitability and infer the impact of hip or knee angle configuration on the muscles' neural drive (27). This technique showed, for instance, a reduction in corticospinal excitability of the VL muscle during MVIC of the knee extensors performed at 100° compared to 75° of knee flexion (7). However, it remains unknown whether this finding remains valid for the other synergist muscles whose recruitment differs during submaximal voluntary contraction (24). It has been suggested that joint position influenced corticospinal excitability likely due to the increase in Ia afferent discharge affecting spinal excitability at long muscle length (7) or impairment of neuromechanical properties requiring different neural control strategies (19). However, the influence of hip and knee angulation on central factors driving muscle recruitment during low-intensity voluntary contraction remains to be precise. This study aimed to examine the influence of hip and knee angle on the neuromuscular function, muscle architecture, and corticospinal excitability of VL mono- and RF bi-articular synergistic muscles of the knee extensors. We first evaluated maximal torque production capacity and muscle length with different knee and hip angle configurations. Secondly, we examined whether knee or hip joint angle influences corticospinal excitability of the VL and RF muscles during submaximal contractions. We hypothesized that increasing knee flexion would: i) increase in maximal force production capacity of the knee extensors with a moderate effect of hip position and ii) alter corticospinal excitability of synergist muscles during submaximal contraction.

Materials and methods

Participants

Sixteen healthy men with no history of neurological disease and no recent lower limb injury participated to this study (age: 25 ± 7 years; mass: 80.0 ± 6.2 kg; height: 177 ± 4 cm). All participants gave their written informed consent prior to the experiment. 114 All procedures conformed to the World Medical Association Declaration of Helsinki (2008) and were approved by the local ethics committee.

Study design

Participants attended the laboratory twice. The first visit was devoted to neuromuscular testing and the second to the ultrasound measurements. In both sessions, four supine positions were randomly tested on the subject’s dominant leg determined as the kicking leg (one subject was left-legged) using knee angle of either 20° or 110° of flexion (0°= fully extended), and hip angle of either 0° or 60° (0° = fully extended) (see Figure 2.2.1.1). Only supine positions were used to avoid the confounding effect of different descending vestibulospinal inputs known to modulate motoneuron excitability (19). The study was conducted using an isokinetic dynamometer (System Pro 4, Biodex Medical System, New-York), and device settings were the same in the two sessions. Participants were lying on their back; the axis of the dynamometer was aligned with the knee joint, and the lever arm was attached 2 cm above the malleoli using a non-compliant strap. The thigh was supported by the seat in positions and $K_{20}H_0$ or using a manufacturer’s device (positions $K_{110}H_{60}$ and $K_{20}H_{60}$). The head was aligned in a neutral position and kept fixed by one experimenter throughout the session.

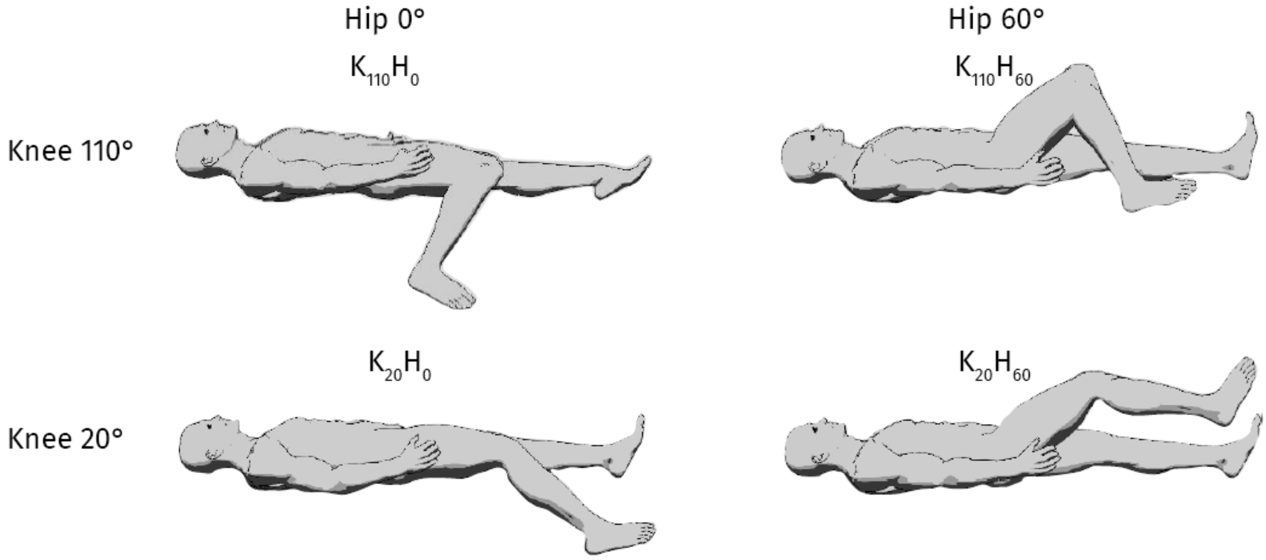

Figure 2.2.1.1 | Description of the different positions tested in the experimental protocol by adjusting knee flexion (20° or 110°) and / or hip flexion (0° or 60°).

Neuromuscular function

For each position, participants performed a warm-up including ten ramping isometric contractions (60% to 100% of the subjects' perceived maximal voluntary contraction). They then performed two maximal voluntary isometric contractions (MVIC), with an additional one if the second was 5% greater than the first. After that, transcutaneous electrical stimulations were carried out on the femoral nerve using a high-voltage constant-current stimulator (model DS7, Digitimer, Hertfordshire, UK). A monopolar cathode-ball (0.5 cm diameter) was pressed into the femoral triangle, and the anode (10 × 5 cm rectangular electrode) was placed on the gluteal fold opposite the cathode. Single pulses (200-μs width) were used at rest to assess neuromuscular function and during a 20% MVIC contraction for motor evoked potential (MEP) normalization. The optimal site of stimulation was determined for each position as the location that evoked the greatest peak twitch and M-wave amplitudes with the same intensity. Once located, stimulation intensity was gradually increased until peak twitch amplitude and M-wave amplitude plateaued and then further increased by 20% to ensure supramaximal intensity. Two single pulses were recorded to determine maximal M-wave amplitude. The ratio between MVIC peak torque and amplitude of the single peak twitch (MVIC/Pt) was used to determine the influence of peripheral factors on torque production capacity (11).

Electromyography recordings

EMG activity of the vastus lateralis (VL), rectus femoris (RF) and biceps femoris (BF) muscles was recorded at a sampling rate of 2 kHz and filtered (10 – 500 Hz) using Acq-Knowledge analysis software (Model MP150, Biopac System, Santa Barbara, CA) using pairs of pre-gelled Ag/AgCl surface electrodes (recording diameter of 10 mm; Mini KR, Controle Graphique S.A., Brie-Comte-Robert, France). Skin was shaved, abraded and cleaned with isopropyl alcohol, then electrodes were taped lengthwise over the middle of the muscle belly with an inter-electrode distance of 20 mm (16). The reference electrode was positioned on the contralateral patella. The root mean square (RMS) value of the EMG was calculated for each muscle over a 100 ms period at the peak torque during the MVIC and normalized by the corresponding M-wave amplitude (RMS_{MVIC}/M). The RMS-EMG of the RF and VL muscles were also calculated over a 100 ms period before the TMS stimulus artefact and normalized to the RMS_{MVIC} to control for muscle activity during MEP recordings (RMS_{MEP} /RMS_{MVIC}). The [(VL-RMS_{MVIC} /M) / (RF- RMS_{MVIC} /M)] ratio was calculated at 20% and 100% MVIC to compare the relative contribution of these two muscles in torque production.

Corticospinal excitability

Transcranial magnetic stimulation (TMS) was delivered with a double-cone coil (110 mm diameter) using a Magstim 200² magnetic stimulator (Magstim, Whitland, Dyfed, UK) during brief (~3 s) weak knee extensor contractions (20% MVIC of the corresponding position). For each position, the optimal coil position was defined as the position that elicited the greatest MEP amplitude in the VL and the RF muscles with the same stimulus intensity (50% maximal stimulator output) and was marked on the scalp to ensure a constant location. The active motor threshold (AMT) was defined as the lowest stimulation intensity that elicited at least 4 over 8 MEP with a distinguishable silent period from background EMG for both muscles (26). Input-output curves were constructed between 90% and 170% of the AMT with incremental steps of 10% to ensure maximal MEP amplitude on the VL and RF muscles (13). Four single pulses per intensity were applied in random order. MEP peak-to-peak amplitude was analysed off-line and normalized to the M-wave amplitude of the corresponding position. Input-output curves were fitted to the Boltzmann sigmoidal function to relate the maximal MEP amplitude (MEPMAX) to the stimulator 176 intensity (S) using the following equation (6):

$$MEP(S) = \frac{MEP_{MAX}}{1 + exp^{\left[\frac{S_{50}-S}{k}\right]}}$$

This function also considered the stimulus intensity required to elicit a MEP that was half of the MEP_{MAX} (S_{50}) and the slope of the curve (k) where its reciprocal ($1/k$) is proportional to the maximum steepness at S_{50}.

Ultrasound recordings

Ultrasound recordings were performed at rest for the VL and RF muscles using B-mode Zonare ultrasound video imaging (Z. One, Zonare Medical Systems Inc. Mountain View, CA, USA). A 5.5-cm (7.5MHz) linear array probe was positioned perpendicular to the dermal surface and oriented along the longitudinal axis of the muscle-tendon unit. The Figure 2.2.1.2 depicted examples of ultrasounds recordings for a representative subject. Images were collected at 50% of muscle length (M_L), calculated as the distance between the proximal and the distal myotendinous junction, determined from the convergence of the deep and superficial aponeuroses. Three images were stored for each muscle to determine off-line fascicle length (F_L) using Kinovea (Kinovea©, 0.8.15 2006 to 2011; Joan Charmant & Contrib, Bordeaux, France). Criteria for storing images were: parallel superficial and deep aponeurosis, and the presence of at least three discernible fascicles. Two to three fascicles were analysed on each image to calculate F_L using the extrapolation method validated in vivo by (4), where *h* is the distance between the intersection point of the visible fascicle with the edge of the image and the superficial aponeurosis:

$$F_L = visible\ fascicle\ length + \frac{h}{\sin(P\alpha)}$$

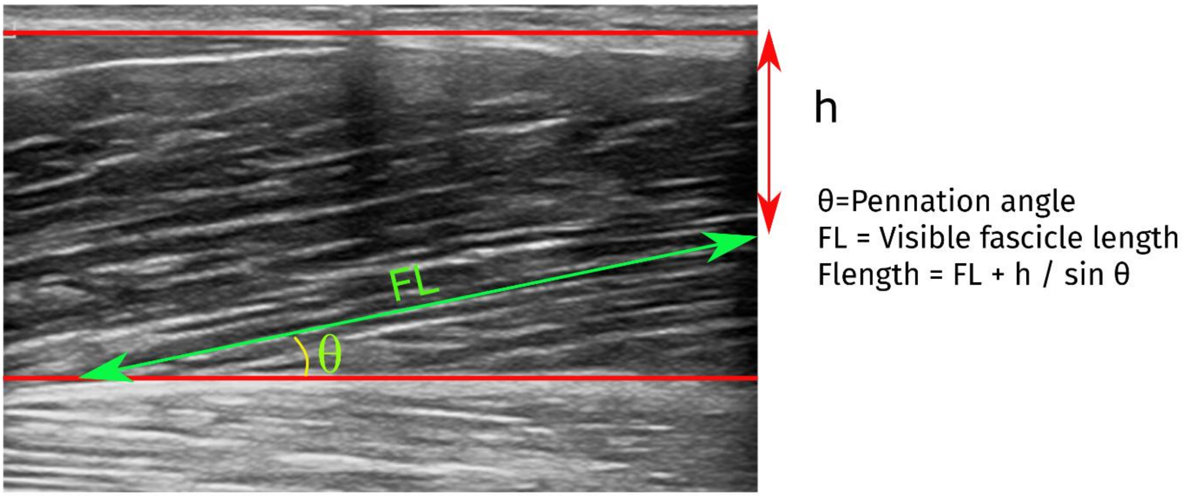

Figure 2.2.1.2 | Example of measurements of muscular architecture for the vastus lateralis (VL) muscles recorded on a typical subject in one of the four different positions. Upper and lower aponeurosis are highlighted in red and green lines indicate the visible fascicle direction.

Statistical analysis

All data are presented as mean ± standard deviation (SD) in text and tables and mean ± 95% confidence interval (CI) in figures. The nature of the distribution was assessed for all variables using the Shapiro-Wilk test. A logarithmic transformation was applied to the data that violated the assumption of normality to ensure the relevant use of parametric testing. Sphericity was checked as appropriate and a Greenhouse-Geisser correction to the degree of freedom was applied when sphericity was violated. One-way ANOVA tested the effect of *position* on MVIC, Pt, MVIC/Pt, M_L and F_L. Two-way ANOVAs tested the effect of *position* and *muscle* on M-wave amplitude, RMS_{MVIC} /M, RMS_{MEP} /RMS_{MVIC} and parameters of the MEP input-output curves (threshold, slope, plateau) or the effect of *intensity* and *position* on [(VL-RMS_{MVIC} /M) / (RF- RMS_{MVIC} /M)] ratio. When significant, the main effect was followed up with a Tukey HSD test. Effect sizes are reported as partial eta squared (ηp^2). Correlations between MVIC and muscle and fascicle lengths were assessed using Pearson's correlation coefficient. Because BF EMG activity log-values were not normally distributed, a Friedman's ANOVA was used to test significant differences. Statistical analyses were performed with Statistica (StatSoft France, version 7.1, STATISTICA) and G*Power 3.1 (10). The significance level was set at 0.05 (2-tailed) for all analyses.

Results

Neuromuscular function

Neuromuscular parameters are presented in Table 2.2.1.1. The ANOVA detected a main effect of position on MVIC ($p < 0.001$; $\eta p^2 = 0.648$) and Pt ($p < 0.001$; $\eta p^2 = 0.762$). Both parameters were greater for positions $K_{110}H_0$ and $K_{110}H_{60}$ compared to positions $K_{20}H_0$ and $K_{20}H_{60}$ (all $p < 0.001$; all dz > 1.184), with no difference between $K_{110}H_0$ and $K_{110}H_{60}$ or $K_{20}H_0$ and $K_{20}H_{60}$ (all $p > 0.081$). No position effect was detected for the MVIC/Pt ratio ($p = 0.528$; $\eta p^2 = 0.048$; see Figure. 2.2.1.3 a). The ANOVA revealed no difference to evoke maximal M-wave amplitude between positions (p = 0.447; ηp^2 = 0.048). The ANOVA detected a main muscle effect on M-wave amplitude (p < 0.001; ηp^2 = 0.905) but neither position nor muscle × position interaction (all p > 0.051; all ηp^2 < 0.175). M-wave amplitude was on average significantly greater for the VL muscle (9.02 ± 5.08 mV) than the RF muscle (3.59 ± 1.84 mV) (p < 0.001; dz = 1.510). Neither main effect, nor a muscle × position interaction was detected for the RMSMVIC /M ratio (all p > 0.060; all ηp^2 < 0.087). The ANOVA detected no significant main effect or interaction on the [(VL-RMS /M) / (RF- RMS /M)] ratio during the 20%MVIC (0.73 ± 0.35) contraction compared to the MVIC (0.96 ± 0.31) (all p > 0.069; all ηp^2 < 0.218). The Friedman's ANOVA detected no difference between position on EMG activity of the BF muscle during MVIC (X^2 = 3.00; p = 0.392).

Table 2.2.1.1 | Neuromuscular (MVIC, Pt and M-wave) and architectural (muscle length and fascicle length) characteristics of the vastus lateralis (VL) and rectus femoris (RF) muscles in the 4 positions (n = 16; mean ± SD). a, b, c Significantly different from position $K_{110}H_0$, $K_{110}H_{60}$ or $K_{20}H_{60}$ respectively. $ Significantly different from RF muscle. One item p < 0.05, two items p < 0.01 and three items p < 0.001.

		Positions			
		$K_{110}H_0$	$K_{110}H_{60}$	$K_{20}H_0$	$K_{20}H_{60}$
MVIC (Nm)		219 ± 74	196 ± 62	131 ± 38 $^{\text{aaa bbb}}$	109 ± 42 $^{\text{aaa bbb}}$
PT (Nm)		51.4 ± 11.7	47.1 ± 11.3	29.5 ± 11.6 $^{\text{aaa bbb}}$	22.5 ± 6.2 $^{\text{aaa bbb}}$
M-wave amplitude (mV)	VL	8.01 ± 4.5 $^{\$\$\$}$	7.05 ± 4.59 $^{\$\$\$}$	8.76 ± 5.23 $^{\$\$\$}$	8.23 ± 6.07 $^{\$\$\$}$
	RF	2.93 ± 1.36	2.68 ± 1.59	3.94 ± 2.41	3.34 ± 1.70
Muscle length (cm)	VL	37.1 ± 3.3	36.0 ± 1.2 $^{\$\$\$}$	33.4 ± 1.7 $^{\text{aaa bbb}}$	31.9 ± 2.1 $^{\text{aaa bbb }\$\$\$}$
	RF	37.2 ± 2.9 $^{\text{ccc}}$	33.8 ± 2.3 $^{\text{aaa ccc}}$	33.3 ± 3.0 $^{\text{aaa ccc}}$	29.8 ± 2.8 $^{\text{aaa ccc}}$
Fascicle length (cm)	VL	14.0 ± 2.0	13.6 ± 2.3	11.2 ± 1.8 $_{\text{aaa bbb}}$	11.5 ± 2.3 $^{\text{aaa bbb}}$
	RF	16.8 ± 2.5	14.4 ± 1.9	12.7 ± 2.1 $^{\text{aaa bb}}$	13.1 ± 3.9 $^{\text{aaa b}}$

Muscle architecture

ANOVA detected significant muscle × position interaction on muscle length (p = 0.001; ηp^2 = 0.289; see Table 2.2.1.1). VL muscle length was greater in positions $K_{110}H_0$ and $K_{110}H_{60}$ compared to positions $K_{20}H_0$ and $K_{20}H_{60}$ (all p < 0.001; all dz 233 > 1.134), without difference between positions $K_{110}H_0$ and $K_{110}H_{60}$ or $K_{20}H_0$ and $K_{20}H_{60}$ (all p > 0.126). The RF muscle was significantly longer in position $K_{110}H_0$ (all $p < 0.001$; all $dz > 1.335$) and shorter in position $K_{20}H_{60}$ (all $p < 0.00$; all $dz > 1.518$) compared to all other positions. The RF muscle length was not different between positions $K_{110}H_{60}$ and $K_{20}H_0$ ($p = 0.843$). VL muscle length was also greater than RF muscle length in positions $K_{110}H_{60}$ and $K_{20}H_{60}$ (all $p < 0.004$; all $dz > 0.831$), but not different for positions $K_{110}H_0$ and $K_{20}H_0$ (all $p > 0.999$). A main position effect was reported for fascicle length for both the VL and RF muscles (all $p < 0.001$; all $\eta p^2 > 0.541$). Both muscles demonstrated greater FL in position $K_{110}H_0$ and $K_{110}H_{60}$ compared to $K_{20}H_0$ and $K_{20}H_{60}$ (all $p < 0.019$; all $dz > 0.693$), and no difference between $K_{110}H_0$ and $K_{110}H_{60}$ or $K_{20}H_0$ and $K_{20}H_{60}$ (all $p > 0.477$). MVIC was significantly correlated to muscle ($r = 0.488$; $p < 0.001$; see Figure. 2.2.1.3 b) and fascicle ($r = 0.529$; $p < 0.001$; see Figure 2.2.1.3 c) lengths.

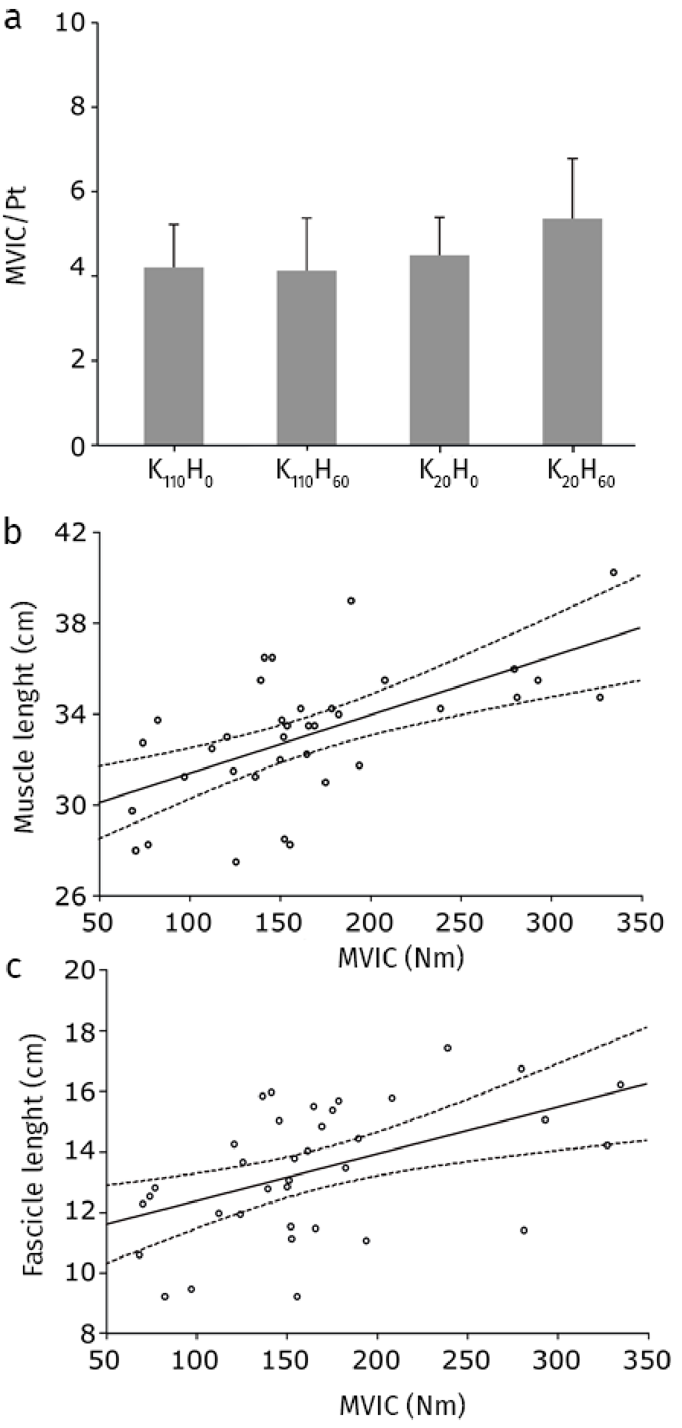

Figure 2.2.1.3 | Ratio between voluntary torque developed during maximal voluntary isometric contraction (MVIC) and peak twitch torque elicited by percutaneous nerve stimulation in each position (a), and relationships between MVIC and mean muscle (b) and fascicle (c) lengths (n = 16; mean ± SD).

Corticospinal excitability

The ANOVA detected no difference between positions in stimulator intensity used to assess the AMT (33 ± 4% maximal stimulator output; $p = 0.739$; $\eta p^2 = 0.027$). The ANOVA detected main effects of muscle and position on the RMS_{MEP}/RMS_{MVIC} ratio (all $p < 0.008$; all $\eta p^2 > 0.407$), but no muscle × position interaction ($p = 0.230$; $\eta p^2 = 0.096$). The RF muscle presented a higher ratio than the VL muscle ($p = 0.008$; $dz = 0.526$). The ratio was also lower for the $K_{20}H_{60}$ position than positions $K_{110}H_{60}$ and $K_{20}H_0$ (all $p < 0.046$; all $dz > 0.284$). Boltzmann-fitted input-output curves recorded in VL and RF muscles using TMS are presented in Figures 2.2.1.4 a and 2.2.1.4 b respectively. Neither main effect (all $p > 0.062$), nor a muscle × position interaction ($p = 0.670$) was detected on normalized MEP amplitude at AMT. The ANOVA detected a significant position effect on maximal MEP amplitude ($p = 0.038$; $\eta p^2 = 0.169$), being

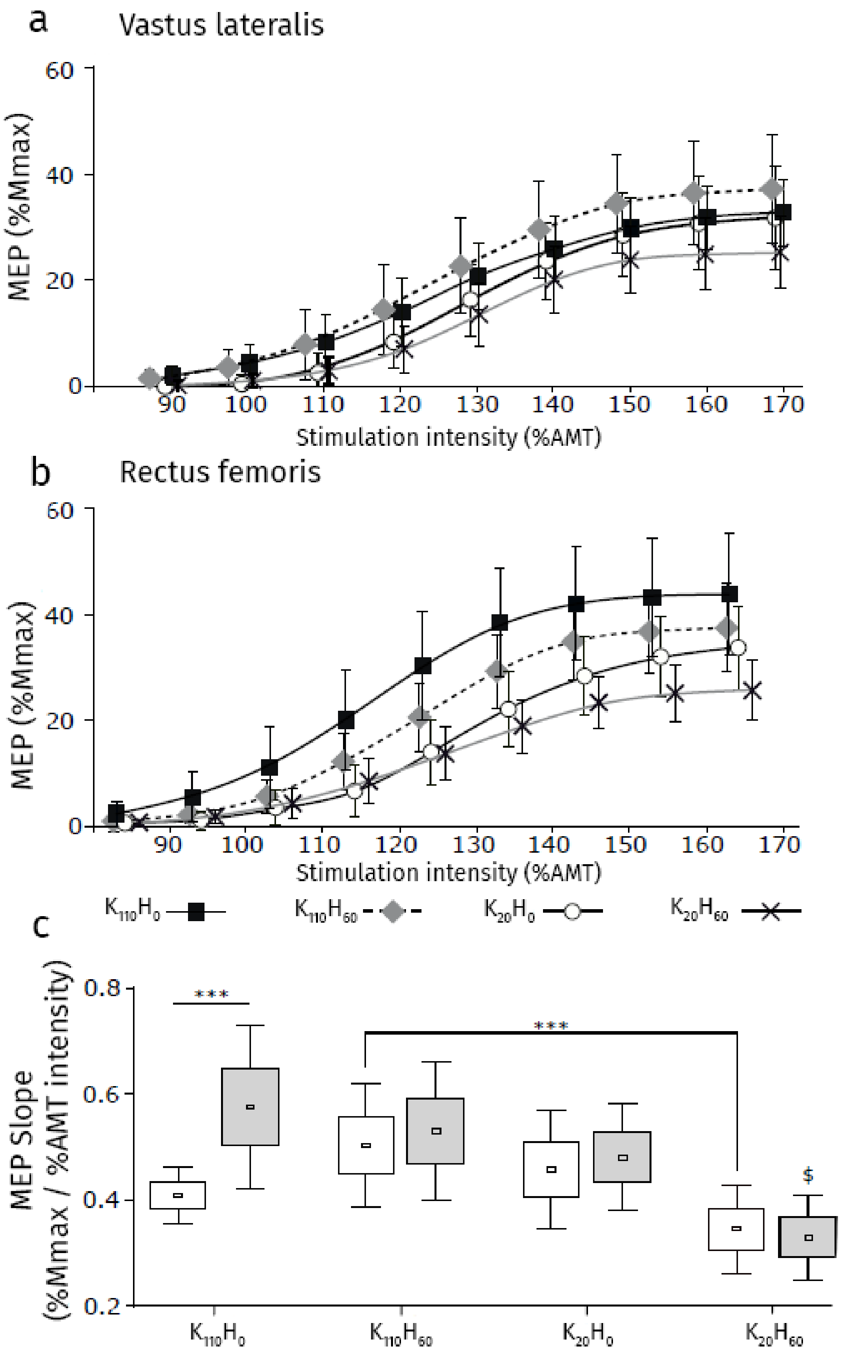

Figure 2.2.1.4 | Input-output curves of mean normalized MEP amplitudes recorded under each condition for the VL (white) (a) and RF muscles (grey) (b) and their corresponding mean slopes (c) (n = 16; mean ± SD). * Denotes significant difference from the position muscle interaction. $ Significantly different than all other positions. One, two or three items denote difference at $p < 0.05$, $p < 0.01$ or $p < 0.001$ respectively.

significantly higher for $K_{110}H_{60}$ than $K_{20}H_{60}$ (p = 0.049; dz = 0.598). No muscle or muscle × position interaction was detected on maximal MEP amplitude (all p > 0.073). The ANOVA showed a significant muscle × position interaction for the slope of the input-output curves (p = 0.006; ηp^2 = 0.237; see Figure 2.2.1.4 c). The RF muscle showed a steeper slope than the VL muscle in position $K_{110}H_0$ (p = 0.001; dz = 0.530). The mean slope of the VL muscle was weaker for the $K_{20}H_{60}$ position than the $K_{110}H_{60}$ position (p = 0.003; dz = 0.105). The RF muscle demonstrated a weaker slope for the $K_{20}H_{60}$ than all other conditions (all p < 0.004; all dz > 0.120).

Discussion

This study sought to examine the influence of hip and knee angle changes on the neuromuscular function, muscle architecture, and corticospinal excitability of knee extensors. The main findings validated our first hypothesis, whereby knee flexion increased maximal force production capacity of the knee extensors, while the hip position has no influence on maximal force production capacity. Furthermore, the present findings also validated our second hypothesis since an increase in knee flexion affected corticospinal excitability of the VL and RF muscles during 20% MVIC of the knee extensors.

In accordance with previous studies (1, 5), our findings demonstrated greater MVIC torque of the knee extensors under knee flexed (i.e., 110° flexion) compared to knee extended position (i.e., 20° flexion). The similar findings regarding the amplitude of the evoked twitch torque (i.e., greater at 110° than 20° knee flexion) resulted in a similar MVIC/Pt ratio between positions (see Figure. 2.2.1.3 a). The constancy of this ratio among the different conditions suggests that contractile properties represent the main determinant in force production capacity of the knee extensors of the different positions (11). Based on the length-tension relationship of isolated muscle fibers developed by Gordon, Huxley, and Julian (14), the use of ultrasonography in this study intended to further examine contractile properties through the influence of muscle and fascicle lengths on the force production capacity of knee extensors. Fascicle length measured in the present study agreed with those reported in a recent study (5). Present data showed an increase in VL muscle length with knee flexion only, while RF muscle length increased in response to either knee flexion or hip extension but remained constant when hip flexion occurred concomitantly to knee extension. These findings suggest, therefore, that despite the same distal insertion of RF and VL muscle on the quadriceps tendon (15), RF muscle length does not influence VL muscle length. The main position effect reported for fascicle length showed a strong effect of knee position with greater lengths for 110° than 20° knee flexion positions for both hip positions considered. Specifically, fascicle length was shorter in position $K_{110}H_0$ than $K_{110}H_{60}$, while no change occurred between positions $K_{20}H_0$ and $K_{20}H_{60}$. In accordance with Xu and colleagues (28), these findings suggest that at 20° knee flexion, muscles remained slack irrespective of the hip angulation used in the present study. Consequently, whenever knee flexion would be fixed at 20°, any change in hip angle between 0° and 60° hip flexion would not influence contractile properties of the knee extensors. Together with the significant linear relation observed between muscle or fascicle lengths and MVIC (Figure 2.2.1.3 b and c), these findings agree with the sliding filament theory (14). Specifically, the greater MVIC developed in the more flexed knee positions would transcribe a more favorable muscle length, increasing the number of actin-myosin bridges that overlap during contraction. Therefore, the lower MVIC torque observed at 20° knee extension could partly result from mechanical deficit due to reduced overlapping of actin-myosin bridges irrespective of hip position. Previous studies also suggested central mechanisms as potential factors altering muscle recruitment and decreasing MVIC of knee extensors in response to the knee (8, 20) or hip (21) extension. Both constant EMG RMS_{MVIC} /M ratio of the VL and RF muscles and similar EMG activity of the BF muscle between the different positions suggest that reduction in MVIC does not result from altered muscle recruitment (24) or different antagonistic co-activation (2). The greater RMS_{MEP}/RMS_{MVIC} ratio reported for RF muscle than VL muscle during the 20% MVIC contraction suggests that neural drive during voluntary contraction of low intensity adapts between synergist mono- and a biarticular knee extensors muscles to control for torque production (24). However, quantification of muscle activity during voluntary contraction remains a limited method to infer the synergistic muscles’ neural control. Single-pulse transcranial magnetic stimulation was applied during 20% MVIC contraction to further investigate the knee extensors' corticospinal excitability. While no difference in AMT was observed in the present study, the slope of the input-output curves, used to characterize the efficiency of motor unit recruitment with the increase in TMS intensity (6), differed slightly between muscles or positions (see Figure 2.2.1.4). Specifically, the slope in position $K_{20}H_{60}$ was lower than in position $K_{110}H_{60}$ for the VL muscle and lower than all other RF muscle positions (see Figure 2.2.1.4 c). The steeper slope observed for the VL and RF muscles in the position $K_{110}H_{60}$ could transcribe a greater gain in corticospinal excitability with increasing stimulation intensity. One could suggest that the greater muscle and fascicle lengths in position $K_{110}H_{60}$ stimulate discharges of static fusimotor axons, which exert an excitatory action on primary and secondary spindle ending (22). The greater plateau value for VL and RF muscles in position $K_{110}H_{60}$ than position K_{20} H_{60} could also mirror greater corticospinal responsiveness (25) and neural sensitivity (12) or a change in the balance between cortical inhibitory and excitatory components in favor of a weaker inhibition (6) of the RF and VL muscles. Taken together, the present findings confirm that muscle length influences corticospinal excitability of the knee extensors (7). However, contrary to maximal voluntary contraction, for which an increase in muscle length reduced corticospinal excitability, our findings suggest an increase in corticospinal excitability during submaximal (20 %MVIC) voluntary isometric contraction with an increase in muscle length. Such difference between maximal and submaximal contractions suggests that neural drive during voluntary contraction of low intensity adapts between synergist mono- and bi-articular knee extensors muscles to control torque production (24). Finally, the slope of the input-output curve was significantly steeper for the RF muscle than the VL muscle only in position $K_{110}H_{0}$, while the slope of the RF muscle in position $K_{20}H_{60}$ was significantly lower than all other position. These findings suggest that the RF muscle's corticospinal excitability is more sensitive to muscle length than VL muscle does. The greater fascicle-to-tendon ratio measured for the RF muscle than the VL muscle (23) suggests that any increase of the RF muscle-tendon unit length would primarily echo on fascicle length and influence muscle spindle discharge compared to a similar change in VL muscle-tendon unit length. However, the investigation of specific change between the VL and RF muscle is beyond the scope of the present study and deserves further measurements to be ascertained. In conclusion, this study suggests that reduction in MVIC, reported for knee extended positions, was mainly due to mechanical disadvantage, in particular short fascicle length leading to an inadequate actin-myosin overlap. In accordance with a recent study (5), the different hip angulations tested in the present study might remain below the slack angle, and therefore would not significantly affect muscle architecture and contractile properties. This discrepancy with precedent findings (9, 21, 24) might be explained by the different range of concomitant change in hip and knee angle used in the present study compared to previous ones. Corticospinal excitability during 20%MVIC increased in response to greater muscle length primarily for the RF muscle and to a lesser extent for the VL muscle. The greater corticospinal excitability with an increase in muscle length could mirror a greater stimulation of the muscle spindles in response to a greater change in muscle and fascicles length.

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2.2.2 Motor cortical excitability changes in preparation to concentric and eccentric movements

Introduction

The performance of coordinated limb movements requires the precise control of muscle activation. The force produced by a muscle is determined by several parameters, including neural activation, muscle length, contraction velocity, and contraction type (isometric, concentric or eccentric). Our movements are accomplished either by concentric (i.e., muscle is contracted and shortens, such as climbing up the stairs) or eccentric (i.e., muscle is contracted and lengthens, such as climbing down the stairs) contractions. These two types of contraction differ with respect to their neuromechanical properties (8, 13, 35, 38). For instance, maximal voluntary force is lower for concentric than eccentric contractions, while the electromyogram (EMG) amplitude is either similar or smaller (8). In addition, previous studies have reported that eccentric compared to concentric contractions present higher risk of injury (36), specific motor unit activation patterns (14), greater force fluctuations (4, 14, 15, 42), and different patterns of cortical activity (23, 40, 41). In particular, a greater BOLD signal intensity was seen in the left primary motor cortex and the right cerebellum and vermis during the performance of concentric contractions (21). Conversely, during the performance of eccentric contractions, greater cortical signal intensities were detected in the right inferior parietal lobe, the pre-supplementary motor area, the anterior cingulate cortex, the right prefrontal, and the left cerebellar hemisphere (23).

Specific neural mechanisms operate at both spinal and supra-spinal levels during eccentric and concentric contractions (8). For example, by using transcranial magnetic stimulation (TMS), it was shown that duration of the silent period recorded after a motor evoked potential (MEP) is shorter during eccentric than concentric contractions in the plantar flexors (9). Also, cortical excitability is greater and short-intracortical inhibition smaller during eccentric compared with concentric contractions (18, 20). Furthermore, it was shown that the slope of the input–output curve and motor threshold were significantly higher during eccentric than concentric contractions, whilst the plateau value of the curve was the same for both tasks (35). Lastly, at the spinal level, a depression of H-reflex amplitude during eccentric contractions has been frequently reported (1, 30).

In this work, we investigated whether the specific neural activations involved in concentric or eccentric muscle contractions could also be observed during the preparation phase of such contractions. It has been proposed that corticospinal excitability, probed by H reflex and/or MEPs amplitude, changes during movement preparation (3, 26). In addition, intracortical recordings in freely moving animals have shown increased frequency of pyramidal cell discharge beginning about 150ms, peaking 50ms before movement onset, and declining 20–30ms after it (12).

Changes in corticospinal excitability during movement preparation can be tested using TMS over the primary motor cortex (M1) by applying a specific protocol named instructed-delay reaction time task (3, 24, 25) in which an informative acoustic cue (Warning cue) is presented in a fixed delay period (e.g. 900, 1000ms) prior to an imperative, proprioceptive signal (Go signal). Towards this goal, we recorded and compared M1 excitability of FDI muscle at different testing times of the preparatory phase of concentric and eccentric contractions. Further, in both contractions, we tested a no-movement (NoMov) condition to disentangle the effects on M1 excitability induced by movement preparation from those induced by the movement preparation linked to the proprioceptive cue.

We predicted an increase in cortical excitability from the early to the late preparatory phase in both contractions. Further, a higher cortical excitability before eccentric movements execution was expected based on previous neurophysiological findings comparing M1 activity during lengthening and shortening contractions (35).

Materials and methods

Participants

Sixteen healthy adults (7 males and 9 females; mean age 24.6 ± 4.7 years) were enrolled in this study. All were right-handed, as determined by the Edinburgh Handedness Inventory (Oldfield, 1971), without previous history of neurological or orthopedic disorders. All participants gave their written informed consent prior to the experiment and did not have any contraindication to transcranial magnetic stimulation (TMS). All procedures were conformed to the World Medical Association Declaration of Helsinki (2008) and were approved by the local ethics committee.

Experimental Protocol

All corticospinal and neuromuscular parameters were collected in a single session (Main experiment). After three weeks, half of the participants (4 males and 4 females; mean age 22.7 ± 3.2 years) took part in a Control experiment. In both experiments, participants randomly performed one set of concentric and one set of eccentric contractions involving the right FDI muscle. They were seated on a comfortable chair and carried out a reaction time task comprising a right index finger movement as a response to a proprioceptive cue that was presented 1s after an acoustic cue. The acoustic cue was produced by a customizable microcontroller board (Arduino Uno). The proprioceptive cue was produced by a custom-made device able to administer a load of 0.5kg in a specific timespan. Therefore, each trial consisted of an acoustic cue (Warning cue), allowing participants to identify the upcoming response, and a proprioceptive cue (imperative Go signal) following the acoustic one after 1s. Immediately after the proprioceptive cue, the load was applied to the index finger of the right hand of the participants. An electromagnet was hooked to the table and was activated to hold up the load linked to the index finger through a cable tie. A personal computer, by means of an ad hoc developed electronic control system, deactivated the electromagnet that consequently released the load. In this way, in the period preceding the Go cue the load did not weight on the participants' finger (Figure 2.2.2.1 a).

The experimental session was preceded by a familiarization period during which participants were instructed to perform either a concentric or an eccentric contraction as soon as they perceived the load pulling the finger. In the case of concentric contraction, the instruction was "to abduct the index finger as fast as possible after the feeling of the load applied to the finger", thus producing a concentric activation of FDI muscle. During the eccentric contraction, participants were requested "to actively resist to the force generated by the weight and carry it down slowly", resulting in an eccentric activation of the FDI muscle (Figure 2.2.2.1 b). During the familiarization period, participants performed each type of contractions for a maximum of 15 trials. During this period participants' motor response was visually monitored by the experimenter, who provided verbal feedback to ensure the correct execution of the movement. The experimental session was divided into two blocks, one for each type of contraction (concentric and eccentric), which were counterbalanced among participants.

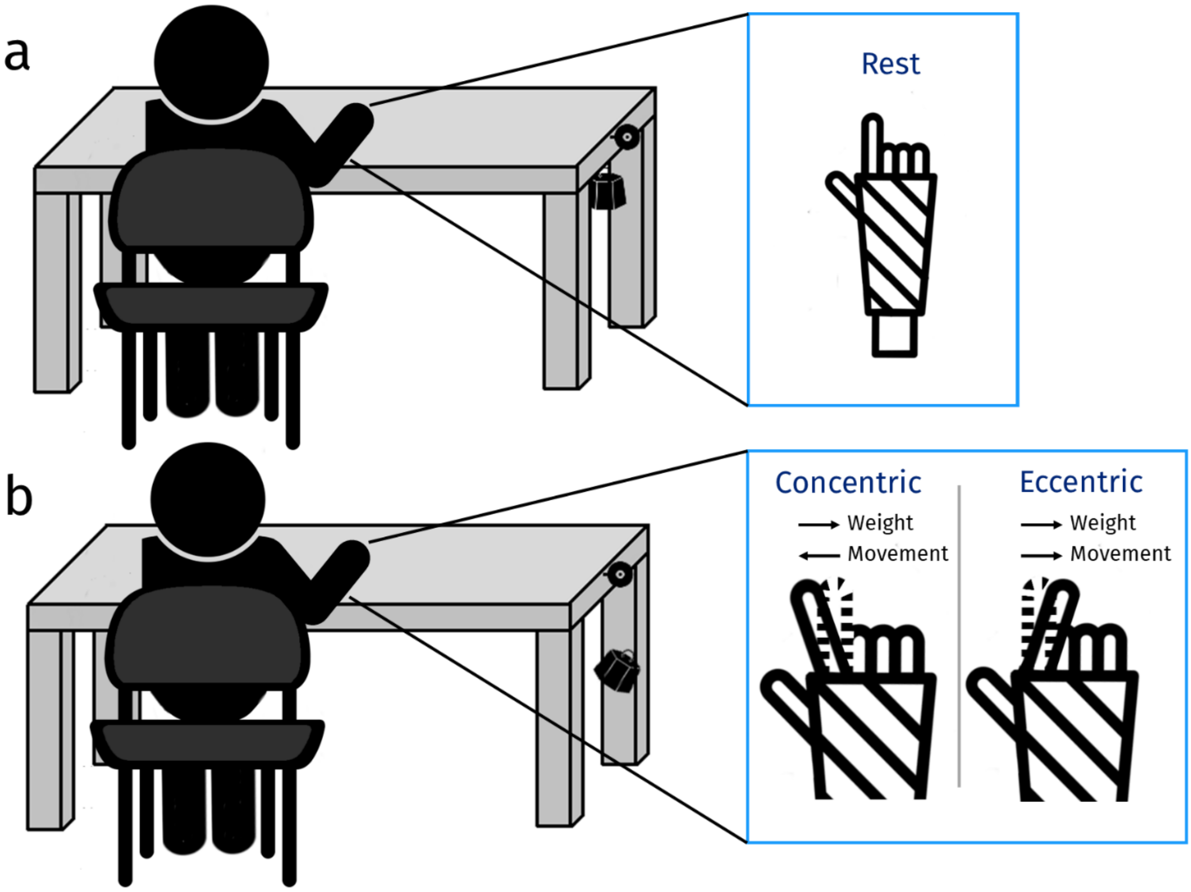

Figure 2.2.2.1 | Experimental set-up. A splint was fixed to the table and the forearm of the participant was bound to the split avoiding any wrist movement but allowing the movement of the index finger. A cable tie was used to link the finger and the load together. An electromagnet was hooked to the downside of the table and hold up the weight of 0.5 kg. A TTL (transistor-transistor logic, 0 - 5V) signal generated by a personal computer was sent to the electronic system controlling the electromagnet in order to release the weight in a specific timespan (Go proprioceptive cue). Participants started the motor task after perceiving the load on the finger, either a concentric movement (abduction of the index finger) or eccentric task (to carry down the weight slowly).

Main experiment

In the Main experiment, we probed the excitability of the primary motor cortex (M1) at three different time points: 150ms (Pre_150) and 50ms (Pre_50) before the Go cue, i.e., during the movement preparation period, as well as 40ms after the Go cue (Post_40), i.e., during the reaction time period (Figure 2.2.2.2 a). These time points were selected in order to evaluate M1 excitability in the period anticipating the EMG onset until 360 ms before it (Leocani et al., 2000). In addition, we tested a no-movement (NoMov) condition interspersed with both set of contractions (concentric and eccentric). In this condition, only the Warning cue was administered, without the presentation of the Go cue, and consequently without the release of the weight. The effects of the NoMov condition on M1 excitability was tested 1s and 40ms after the Warning cue (late preparatory phase), at the time point corresponding to Post_40 condition (Figure 2.2.2.2 b).

Control experiment

In order to investigate possible differences in the early preparatory phases of concentric and eccentric contractions (Lebon et al., 2016, 2019), we retested 8 participants out of 16 at two time points: 300ms (Pre_300) and 150ms (Pre_150) before the Go cue (Figure 2.2.2.2 c).

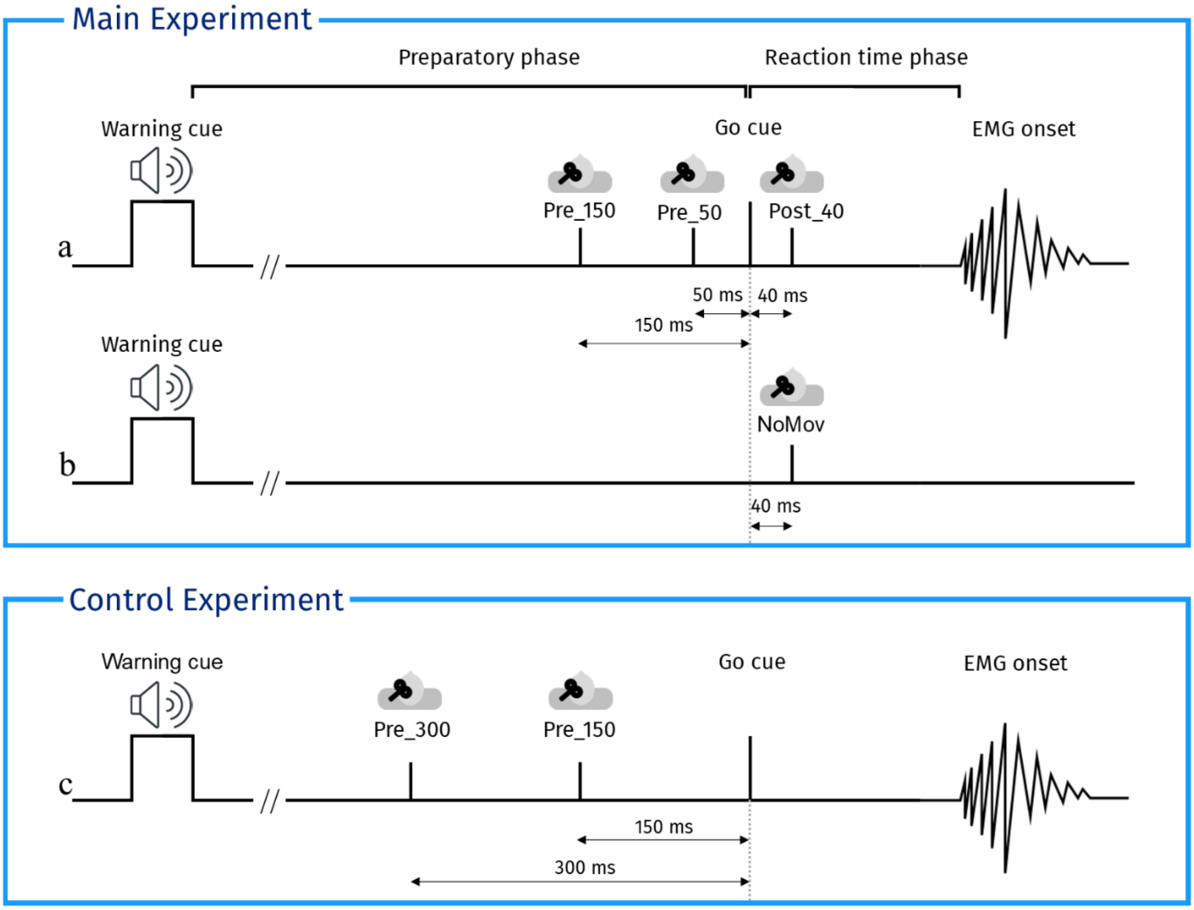

Figure 2.2.2.2 | Experimental procedure. a) In the Main experiment, M1 excitability was assessed at 150 ms (Pre_150) and 50 ms (Pre_50) before the Go cue, during the preparatory phase, and 40 ms (Post_40) after the Go cue, during the reaction time phase. b) M1 excitability was also assessed 1 s and 40 ms after the Warning cue, without any Go cue (NoMov condition), in correspondence to the late preparatory phase. c) In Control experiment, M1 excitability was assessed at 300 ms (Pre_300) and 150 ms (Pre_150) before the Go cue.

Electromyographic (EMG) recording

EMG was recorded with silver disc surface electrodes placed on a tendon belly arrangement over the bulk of the right FDI muscle. We calculated the root mean square (RMS) of the EMG signals to analyze muscle activity during the motor response and to measure the reaction time (EMG-RT) of the motor task, defined as "the elapsed time from the stimulus onset to the appearance of the EMG activity just preceding the actual finger or limb movement" (5). EMG signals were amplified and filtered (20Hz to 1kHz) with a D360 amplifier (Digitimer). The signals were sampled at 5000 Hz, digitized with a laboratory interface (Power 1401, Cambridge Electronic Design, Cambridge, UK), and stored on a personal computer for display and later offline data analysis of RMS of the EMG activity and the analysis EMG-RT.

Transcranial magnetic stimulation (TMS)

Single-pulses were delivered using a Magstim 200 stimulator (Magstim, UK) with a monophasic current waveform connected to a figure-of-eight-shaped coil (external diameter of each loop, 9 cm) held tangentially to the scalp. The center of the junction of the coil was placed over the hand area of the contralateral M1 at the optimal position (hot spot) to elicit MEPs in the right FDI, with the handle pointing backwards and <45° away from the midline. With this coil orientation, the induced current flowed in an anterior–medial direction approximately perpendicular to the central sulcus. The optimal coil location was searched by slightly moving the coil over the left M1 area until MEPs of maximal amplitude and lowest threshold in the right FDI muscle were elicited. The exact coil position was marked by an inking pen to ensure an accurate positioning of the coil throughout the experiment. The intensity of stimulation was adjusted to obtain MEPs of about 1mV in amplitude. Subsequently 20 stimulations at rest were recorded and used as baseline. In the Main experiment a total of 120 MEPs were recorded: 15 for each condition (Pre_150; Pre_50; Post_40 and NoMov) in both contraction conditions. In the Control experiment 60 MEPs were recorded: 15 for each condition (Pre_300; Pre_150) in both contraction conditions.

Data and statistical Analyses

Trials for which the mean EMG activity before the Go cue was larger than the mean + SD of the EMG activity recorded during rest were rejected (less than 2%). Further, trials in which reaction time was too short or too long with respect to subjective response (i.e., mean ± twice the standard deviation) were also rejected (less than 1%) to avoid any incorrect trials. Lastly, MEPs with outlier values (mean ± twice the standard deviation) were removed from further analysis (less than 5%).

Data recorded in both Main and Control experiments were normally distributed according to the Shapiro–Wilk test. In the Main experiment, we examined changes in M1 excitability by applying a two-way repeated-measures ANOVA with contraction (concentric vs. eccentric) and time (Pre_150, Pre_50 and Post_40) as within-subject factors. For the same parameter, we also applied a two-way repeated-measures ANOVA with condition (Mov vs. NoMov measured at Post_40) and contraction (concentric vs. eccentric) as within-subject factors. Then a two-tailed paired t-test was run to assess differences for contraction (concentric vs. eccentric) in the NoMov condition. EMG-RT or RMS differences between the two contractions (concentric vs. eccentric) were assessed by means of a two-tailed paired t-test. Concerning the Control experiment, to assess the effects of contraction (concentric vs. eccentric) and time (Pre_300 and Pre_150) on M1 excitability in the early phase of movement preparation, we used a two-way within-subjects repeated-measures ANOVA. All significant interactions were further examined with Newman-Keuls post-hoc comparisons. The level of significance was set at $p < 0.05$. Statistical analysis was performed with Statistica software.

Results

All participants successfully completed the experiment. The mean MEP amplitude attained during the TMS at rest was 0.990 ± 0.185mV. The mean RMS values of the EMG activity during motor response were greater for the concentric compared to the eccentric contraction (respectively, 0.769 ± 0.425mV and 0.665 ± 0.404mV; t = 4.35; p < 0.001). The mean value of EMG-RT was not different between concentric and eccentric contractions (respectively, 194 ± 31ms and 189 ± 31ms; t = 1.24; p = 0.233).

Main experiment

Figure 2.2.2.3 a shows the average values (± SE) of MEPs amplitude recorded at different times in the eccentric and the concentric conditions. A significant interaction between contraction and time was shown by RM-ANOVA ($F_{(2,30)}$ = 8.462; p = 0.001; η2 = 0.361). For the eccentric contraction, post hoc analysis showed that MEPs in Pre_50 were significantly lower with respect to those recorded in Pre_150 (p = 0.039) and Post_40 (p < 0.001). In addition, MEPs recorded at Pre_150 were significantly lower than MEPs recorded at Post_40 (p < 0.001). No significant difference in MEP values was observed for the concentric

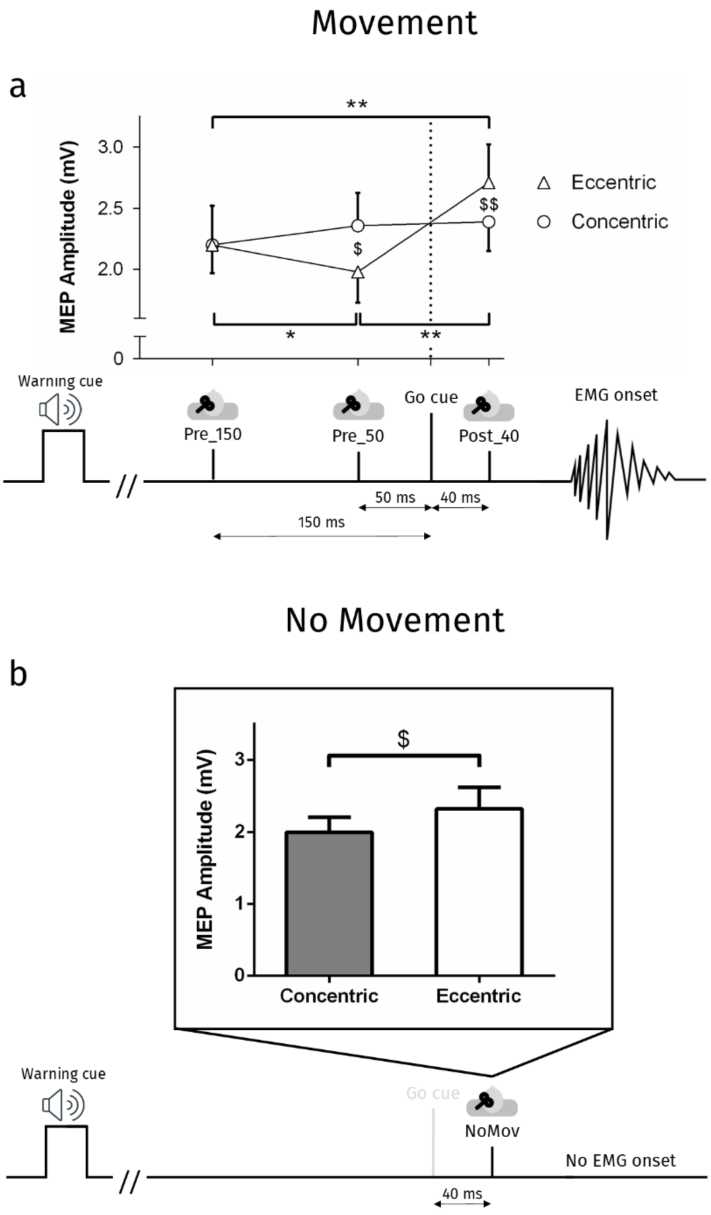

Figure 2.2.2.3 | Main experiment results. a) Mean values (±SE) of MEPs amplitude recorded in the main experiment at different times during concentric (circles) and eccentric (triangles) contractions. The time were 150ms (Pre_150) and 50ms (Pre_50) before the Go cue, i.e., during the preparatory phase, and 40 ms (Post_40) after the Go cue, i.e., during the early reaction time phase (see Figure 2.2.2.2a). b) The mean values (±SE) of MEPs recorded in the no-movement condition for concentric (grey) and eccentric (white) contractions tested 1 s and 40ms after the Warning cue (NoMov condition, see Figure 2b). The symbol * indicates significant differences within the eccentric contraction, whilst the symbol $ indicates significant differences between the two contraction types. One symbol indicates p level < 0.05, two symbols indicate p level < 0.01.

contraction (p always > 0.05). Further, whilst in Pre_150 MEPs' amplitude was similar between concentric and eccentric contractions ($p = 0.523$), significant differences were observed between them in Pre_50 ($p = 0.027$) and in Post_40 ($p = 0.009$).

We also tested M1 excitability during eccentric and concentric contractions in the no-movement condition (Figure 2.2.2.3 b). RM-ANOVA on MEP amplitudes showed a significant main effect of condition, MEPs in the Mov condition was higher than those in the NoMov condition ($F_{(1,15)} = 9.153$; $p = 0.009$; $\eta 2 = 0.380$), and a significant main effect of contraction, with eccentric MEPs higher than concentric MEPs ($F_{(1,15)} = 5.550$; $p = 0.033$; $\eta 2 = 0.271$). No condition x contraction interaction was observed ($F_{(1,15)} < 0.001$; $p = 0.989$; $\eta 2 < 0.001$). Note that a separate analysis for the NoMov condition only (Figure 2.2.2.3 b) revealed significantly greater MEPs amplitude for the eccentric than the concentric contractions ($t = 2.293$; $p = 0.037$).

Control experiment

Figure 2.2.2.4 shows the mean values of MEPs amplitude recorded at 300ms and 150ms before the Go signal. ANOVA showed a significant main effect of time ($F_{(1,7)} = 21.248$; $p = 0.002$; $\eta 2 = 0.752$). MEPs amplitude significantly increased when approaching the Go cue for both contractions ($p = 0.002$). No main effect of contraction ($F_{(1,7)} = 0.158$; $p = 0.703$; $\eta 2 = 0.022$), neither contraction x time interaction ($F_{(1,7)} = 0.114$; $p = 0.745$; $\eta 2 = 0.016$) were observed.

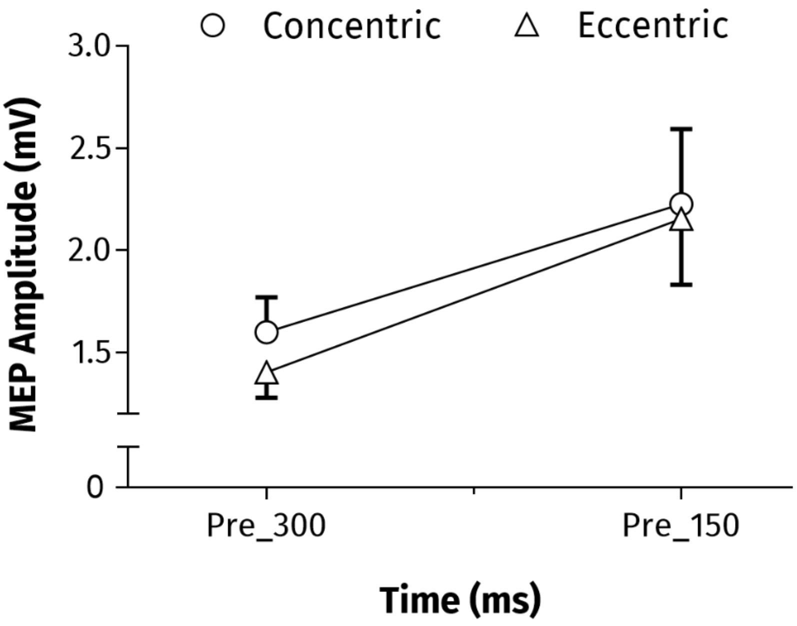

Figure 2.2.2.4 | Control experiment results. The mean values (±SE) of MEPs recorded in Control experiment at different time points during concentric (circles) and eccentric (triangles) contraction. The time points were 300ms (Pre_300) and 150ms (Pre_150) before the Go cue (see Figure 2.2.2.2 c). The symbol ** indicate significant difference between time points for both contractions ($p < 0.01$).

Discussion

The present study focused on M1 excitability during the preparatory phase of concentric and eccentric muscle contractions in an instructed-delay reaction time task. Precisely, we tested M1 excitability at different time points ranging from 300ms before up to 40ms after a Go signal. A common significant increase in M1 excitability from 300ms to 150ms before the Go signal was observed for both contractions. Interestingly, significant changes in M1 excitability in the time interval around the Go signal (-50ms to +40ms) were only revealed in eccentric contraction. Indeed, there was a significant decrease of M1 excitability immediately before the Go cue (Pre_50) and a significant increase 40ms after it (Post_40) with respect to the MEPs recorded at Pre_150.

Previous studies have shown differences in corticospinal excitability between concentric and eccentric contractions during movement execution. Specifically, cortical excitability is greater and short-intracortical inhibition smaller during eccentric compared with concentric contractions (18, 20). In addition, the slope of the input–output curve and motor threshold were significantly higher during eccentric than concentric contractions, whilst the plateau value of the curve was the same for both tasks (35). Here, we replicated these results, finding an increased corticospinal excitability during eccentric contractions in the Post_40 condition. Interestingly, we found the opposite during motor preparation (Pre_50); i.e., a decrease in the corticospinal excitability for the preparation of an upcoming eccentric contraction compared with a concentric contraction.

The significant decrease of M1 excitability observed only during eccentric contraction at 50ms preceding the imperative cue could be due either to a reduction in excitation or an increase in inhibition. This because the MEPs amplitude obtained during all preparatory phases are already greater than at rest. However, corticospinal excitability changes occurring during movement preparation were associated with inhibition processes, especially when different motor response may be selected (11, 16, 34). The functional role of changes in M1 excitability before movement initiation has been subject of considerable debate (10). Among the different hypotheses proposed in the literature, one deals with the regulation of response initiation: decrease in corticospinal excitability serves to prevent premature movement, while preparatory activity unfolds across the cortex (11, 16). Another view is that preparatory inhibition may serve to modulate the gain of the motor system, a way to promote rapid action execution (16). Both hypotheses can fit very well to explain the decrease of M1 excitability preceding an eccentric contraction. Indeed, it has been demonstrated that eccentric movements require a greater cortical control to be prepared, planned, and performed with respect to concentric movements (13, 14, 42). Several studies have reported a larger recruitment of brain areas during eccentric muscle actions (32). This greater extended motor network (including the primary, secondary sensory and association cortices) may be one control strategy for both activating high-threshold motor unit recruitment and lowering discharge rate of activated motor units occurring during eccentric muscle actions (21, 29). Conversely, the lack of M1 excitability modulation in concentric contraction supports the idea that mechanisms dealing with the regulation of response initiation or the rapid action preparation and execution are not necessary, at least when the task is simple and an action selection is not required, as in our paradigm.

Interestingly, we did not find any difference in the reaction times between the two types of contractions. This finding indicates that the two neural strategies did not have a functional impact, being well-adapted to the task constraints. It is necessary noticing that the reaction time used in this study is considered an EMG-RT defined as the elapsed time from the stimulus onset to the appearance of the electromyographic activity just preceding the actual finger or limb movement (5). This EMG-RT may be considered as the premotor component of the reaction time differing from motor component because it is based upon the start of electromyogram instead to the onset of movement (2, 5).

MEPs recorded at Post_40 in eccentric condition were higher than MEPs recorded at the same time point during concentric contractions. This result does not refer to an increase of force in the eccentric condition with respect to the concentric one; firstly, because the same load was applied in both the contraction types, and secondly a similar result concerning M1 excitability, evaluated at the same time point, was also found in the NoMov paradigm. Moreover, we found that RMS of EMG signals was significantly lower in eccentric compared to that measured in concentric contractions. This result is in line with previous works showing that eccentric contractions require the activation of fewer motor units with respect to concentric ones when the same force is used (33). It was also reported in studies on lower limb (28) as well as on upper limb muscles (27), showing lower EMG amplitude during eccentric contraction. Previous studies have suggested that the increased cortical excitability during eccentric contraction resulted in extra-excitatory descending drive to compensate for spinal inhibition (9, 18) and that corticospinal pathway could specifically modulate the Ia motoneuron synaptic transmission during muscular lengthening (17).

The significant increase of M1 excitability observed during the eccentric contraction, when the motor response has been just planned but not executed (40 ms after the Go signal), could also reflect an increase of the activity of the attentional processes involved in the eccentric contraction. Several lines of evidence point to different cortical mechanisms in the execution of concentric and eccentric contractions; larger cortical excitability, greater involvement of brain areas, and different modulations of intracortical and interhemispheric coordination have been proposed (19). The result we found might be attributed to a different strategy of motor processing and to a higher level of cognitive demand required during eccentric contractions in comparison with concentric ones (13). This assumption is supported by Kwon et al. (23), who investigated the functional difference between eccentric and concentric contractions through a fMRI study, showing that lengthening and shortening muscle contractions induced different patterns of cortical activity. Their findings revealed that cortical areas associated with motor performance were mainly activated in eccentric contractions, including pre-supplementary motor area, prefrontal cortex and anterior cingulate cortex, areas known to be involved in the attention ability (39), that together can induce an increase in arousal levels. Also, it should be considered that all the participants familiarized with the two motor task conditions before the experiment and, therefore, the level of M1 excitability measured in the NoMov condition can be thought as related to the motor strategy adopted in preparation to the forthcoming motor task. Interestingly, also in the NoMov condition M1 excitability in eccentric contraction was higher than that evaluated in the preparatory phase of the concentric contraction. However, the amplitude of MEPs was lower with respect to those recorded in the Movement condition in both contractions. The latter finding might be explained by an effect of temporal expectation on M1 excitability. Indeed, an effect of temporal expectation on M1 excitability was found by our group in a recent study on action observation (31). Therefore, following this notion, we can suppose that the lack of an imperative cue at an expected time point can induce, with time, a reduction of M1 excitability in both concentric and eccentric condition. However, we cannot exclude that the lack of the proprioceptive cue in NoMov condition could reduce the excitability at the spinal level. Therefore, we can assume that the MEP amplitude in NoMov condition refers, almost exclusively, to a cortical contribution, whilst in movement condition MEP amplitude could be the result of peripheral and central contributions.

To this propose, we cannot rule out possible changes occurring in the spinal cord that resulted in changes to MEP amplitudes in the Post_40 condition. Indeed, the increased MEPs values 40 ms after the imperative cue could be due to muscle spindles’ contribution. Previous studies have shown that electrical and mechanical stimuli increase MEPs' amplitude in the stimulated muscles (6, 22, 37). However, in the present study, the imperative-proprioceptive cue was present in both eccentric and concentric conditions, leading to similar muscle spindles response in the two conditions, which cannot explain the present findings. Moreover, the modulation exerted by muscle spindles on MEP amplitude has not been observed after passive mobilization (7), which has provided evidence of marked potentiation in MEP amplitude during passive muscle shortening and some evidence of reduced MEP amplitude during passive muscle lengthening.

In conclusion, we showed a time-specific modulation of M1 excitability in the preparatory phase to an eccentric FDI muscle contraction. Future work is required to evaluate which is the role of this modulation in the regulation of movement initiation and setting of the response gain, with the goal to explain the neural mechanisms controlling those complex movements mainly based on eccentric contractions.

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