

Changes in Brain Resting-State Functional Connectivity Following a 2-Week Upper Limb
Immobilization in Female Participants.

Thesis by
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Abstract

Following limb immobilization, the loss in muscle strength typically surpasses the loss in muscle mass. This is most likely caused by the involvement of other factors, such as neural mechanisms, in the loss in force production. These neural mechanisms include, but are not limited to, changes occurring in the brain during limb immobilization. These brain-related changes have not been fully described, and only a few studies have aimed at quantifying the functional changes that occur in the brain during limb immobilization using functional magnetic resonance imaging (fMRI). The connecting thread between the main findings of each study is the changes in activation and connectivity occurring in the sensorimotor network, which seems to be most affected by extended periods of immobilization. The aim of this study was thus to offer more details regarding the resting-state functional connectivity (rs-FC) of the different cortical, subcortical, and cerebellar regions of the sensorimotor network. Twelve female participants underwent fMRI scans before and after completing a two-week elbow immobilization period. The main findings suggest a profound impact of immobilization on the cerebellum, which underwent three significant changes in connectivity. A seed placed in the immobilized upper-limb representation of the cerebellum had a decrease in connectivity with a region of the left temporal lobe (middle temporal gyrus) and an increase in connectivity with the bilateral supplementary motor area. In contrast, a seed placed in the ventral premotor cortex associated with the non-immobilized limb increased in connectivity with the non-immobilized upper-limb cerebellar representation. The only significant change that did not involve the cerebellum was an increase in connectivity between the immobilized motor thalamus and posterior insula. Overall, these changes seem to involve regions mainly responsible for error detection, coordination, and motor learning. These findings show that muscle strength is not the only factor affected and suggest the importance of relearning motor patterns following limb immobilization.

Résumé

L'immobilisation d'un membre engendre des pertes de force et de masse musculaire aussi tôt que trois jours après le début de l'immobilisation. Toutefois, la perte de force excède typiquement la perte de masse, suggérant la contribution d'autres facteurs à la réduction de la capacité à produire de la force. Parmi ces facteurs, des changements cérébraux pourraient aussi contribuer à diminuer la production de la force. Ces changements cérébraux ne sont pas bien compris, mais le système sensorimoteur semble être le plus affecté. L'imagerie par résonance magnétique fonctionnelle est une technique qui permet de quantifier la connectivité fonctionnelle au repos, une mesure de la synchronicité des décharges spontanées de différentes régions du cerveau au repos. L'objectif de cette étude était donc de déterminer si la connectivité fonctionnelle au repos du cerveau était affectée par une immobilisation du coude pour une période de deux semaines. Douze participantes ont participé à l'étude. Les séquences d'imagerie par résonance magnétiques ont été collectées avant et après avoir complété la période d'immobilisation. Une analyse basée sur des régions d'intérêt sensorimotrices corticales, sous-corticales et cérébelleuse a été effectuée. Les changements causés par l'immobilisation impliquaient principalement le cervelet. La région motrice de ce dernier du côté immobilisé (lobule VIIla) a perdu sa connectivité avec une région du lobe temporal gauche, alors qu'elle a gagné en connectivité avec l'aire motrice supplémentaire bilatéralement. Une région prémotrice associée avec le bras non-immobilisé et une région motrice du cervelet du même bras (lobule VIIb) ont aussi gagné en connectivité. Une région du thalamus du côté immobilisé a gagné en connectivité avec le cortex insulaire postérieur immobilisé. En somme, les changements de connectivité se sont principalement produits dans des régions du cerveau typiquement associées avec la détection d'erreur et l'apprentissage moteur, suggérant que la réorganisation fonctionnelle peut prendre place en aussi peu que deux semaines et affecter principalement des régions du cerveau impliquées dans la coordination du bras immobilisé.

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Preface and Contribution of Authors

Julien Clouette conducted this study as part of his master's thesis and was responsible for participating in data collection, preprocessing, analyzing and interpreting the neuroimaging data, writing the thesis, and preparing the manuscript. Alexandra Potvin-Desrochers contributed to data collection, the neuroimaging data preprocessing, analysis and interpretation, and reviewing the manuscript. Freddie Seo was responsible for designing the study, leading data collection, recruiting subjects, processing and analyzing the data unrelated to neuroimaging, and reviewing the manuscript. Tyler Churchward-Venne contributed to designing the study and received funding for this project. Caroline Paquette supervised the writing of this thesis and manuscript, contributed to designing the study, and to data preprocessing, analysis, interpretation.

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List of Abbreviations

BET: Brain Extraction Tool
BOLD: Blood-Oxygen-Level-Dependent
CAF: C-Terminal Agrin Fragment
CNS: Central Nervous System
CSF: Cerebrospinal Fluid
DMN: Default Mode Network
dPMC: Dorsal Premotor Cortex
FEAT: FMRIB Expert Analysis Tool
FLIRT: FSL's Linear Image Registration Tool
fMRI: Functional Magnetic Resonance Imaging
FSL: FMRIB Software Library
ICA: Independent Component Analysis
IGF-1: Insulin-Like Growth Factor 1
M1: Primary Motor Cortex
MNI: Montreal Neurological Institute
MRI: Magnetic Resonance Imaging
MTG: Middle Temporal Gyrus
NCAM: Neural-Cell Adhesion Molecule
NMJ: Neuromuscular Junction
PMC: Premotor Cortex
PNS: Peripheral Nervous System
ROIs: Regions of Interest
rs-FC: Resting-State Functional Connectivity
TMS: Transcranial Magnetic Stimulation
S1: Primary Somatosensory Cortex
SMA: Supplementary Motor Area
VA: Voluntary Activation
VL: Ventrolateral Nucleus of the Thalamus
vPMC: Ventral Premotor Cortex
WM: White Matter

Chapter 1: Introduction

Limb immobilization is often used as a rehabilitation technique. Nevertheless, it still imposes an important burden on individuals undergoing immobilization protocols. Immobilization not only makes activities of daily living harder to perform, but it also has immediate and lingering effects on the muscle. Indeed, the adaptability of skeletal muscles makes them one of the most plastic organ in the human body (Frontera & Ochala, 2015). This can have a positive impact and allow for an increase in force output in response to strength training, but it can also cause a decrease in strength following disuse caused by immobilization (Booth, 1982; Frontera & Ochala, 2015). Counterintuitively, studies have shown that, in the case of disuse, the loss in strength can greatly surpass the loss in muscle mass – muscle atrophy – suggesting that mechanisms other than muscle atrophy contribute to a loss in contractile force (Booth, 1982; Frontera & Ochala, 2015). These mechanisms include both musculoskeletal factors such as changes in single fiber characteristics and tendon stiffness, as well as neurological factors such as muscle denervation, damage to neuromuscular junctions, and supraspinal – brain-associated – changes (Marusic, Narici, Simunic, Pisot, & Ritzmann, 2021). While the occurrence of neurological changes is well-established, the complete underlying mechanisms are not fully understood (Campbell et al., 2019).

In order to better characterize those supraspinal changes occurring during and after limb immobilization, a few studies focusing on the brain have used functional magnetic resonance imaging (fMRI) (Burianová et al., 2016; Farthing et al., 2011; Garbarini et al., 2019; Langer, Hänggi, Müller, Simmen, & Jäncke, 2012; Lissek et al., 2009; Newbold et al., 2020). fMRI is a brain-imaging technique with high spatial resolution that allows to quantify activation of specific brain regions as well as measure connectivity between different regions (Logothetis, 2008). Although the novelty of using fMRI in limb immobilization protocols has prevented the results of these studies from being reproduced at all, some of the different protocols exploited

so far seem to produce similar observations. First, a decreased activation of the disused primary sensorimotor regions has been observed in a variety of fMRI protocols: during hand contractions, motor imagery, and electrical stimulation of the finger (Burianová et al., 2016; Garbarini et al., 2019; Lissek et al., 2009). Moreover, in the only study looking at connections between regions of the brain at rest, a disconnection of the same disused regions from the rest of the sensorimotor network was observed (Newbold et al., 2020). This study was nevertheless performed on only three participants. Furthermore, no study in the current literature has used fMRI to assess brain changes in female specifically, despite the fact that they are known to be more affected by immobilization protocols, undergoing more important losses in strength (Deschenes, McCoy, Holdren, & Eason, 2009; Deschenes, McCoy, & Mangis, 2012; Yasuda, Glover, Phillips, Isfort, & Tarnopolsky, 2005).

fMRI is a technique that allows to measure connections in the brain at rest, known as resting-state functional connectivity (rs-FC). rs-FC measurements are performed at rest as opposed to being performed during a specific cognitive or motor task, which allows to make observations regarding the spontaneous activation occurring in different regions of the brain (Fox & Greicius, 2010; Greicius, Supekar, Menon, & Dougherty, 2009). The timing of this spontaneous activation in turn provides information regarding functional connections between the regions that discharge simultaneously (Fox & Greicius, 2010; Greicius et al., 2009).

The purpose of the present study was to quantify the changes in rs-FC that occur over a 14-day upper-limb immobilization period in female participants. The hypothesis-driven analysis focused on cortical regions, but also included exploratory subcortical and cerebellar regions, which have been somewhat left out from the studies that make up the current literature on the subject. This type of analysis provides insight regarding the mechanisms occurring in the brain during the immobilization of a limb. A clearer understanding of the changes occurring

in the brain during immobilization ultimately has the potential to help prevent some of the negative consequences associated with this process.

Although the current state of the literature is very limited regarding changes in rs-FC caused by limb immobilization in healthy individuals, a general hypothesis of the changes expected was formulated based on the evidence available:

- (1) Based on the data reported by Newbold et al. (2020) and Garbarini et al. (2019), it is expected that disused regions such as the primary motor cortex (M1), the primary sensory cortex (S1), and the upper-limb representation of the cerebellum will be more strongly connected following two weeks of limb immobilization.
- (2) Also based on the data from Newbold et al. (2020), these disused regions will be expected to become disconnected from the rest of the sensorimotor network.

These two hypotheses thus suggest that a segregation of the disused regions from the rest of the brain is likely to occur as a result of the immobilization period.

Chapter 2: Literature Review

2.1 Limb Immobilization

Whether it aims at healing an injury such as a wrist fracture (Sabbagh, Morsy, & Moran, 2019), or is used to diminish the unilateral motor consequences of a stroke (Furlan, Conforto, Cohen, & Sterr, 2016), limb immobilization can be used as a therapeutic strategy in people from all walks of life. Despite its widespread and well-known use, however, immobilization imposes an important burden on people who must resort to this strategy. Limb immobilization greatly limits physical activity (Beckenkamp, Lin, Engelen, & Moseley, 2016) and makes tasks of daily living much more complicated.

2.1.1 Musculoskeletal Consequences of Limb Immobilization

Limb immobilization is well-known for causing long-lasting changes in bone density and muscle strength (Booth, 1982; Campbell et al., 2019; Marusic et al., 2021). Studies have observed a decrease in bone density with limb immobilization or bed rest in both animals and humans (Bloomfield, 1997; Demirbag, Ozdemir, Kokino, & Berkarda, 2005; Friedman, Zhang, Wayne, Farber, & Donahue, 2019; Stewart, Werpy, McIlwraith, & Kawcak, 2020), which can introduce a fragility that can last for more than 6 months following the immobilization (Bloomfield, 1997). At the level of the muscle, the decrease in mass systematically occurs, even if the exact mechanism through which this happens is still unclear. It was initially thought that the IGF-1-Akt-mTOR pathway, which plays an important role in muscle protein synthesis and human growth, was inhibited in immobilization, causing the muscle atrophy (Gao, Arfat, Wang, & Goswami, 2018). However, markers of activity of this pathway do not seem to vary during prolonged immobilization (de Boer et al., 2007). Thus, the exact mechanism causing muscle atrophy is currently not fully understood. The overall consequence, nevertheless, is a decreased rate of muscle protein synthesis, most likely paired with an increased rate of muscle protein breakdown, ultimately leading to a loss in muscle mass (McGlory, van Vliet, Stokes, Mittendorfer, & Phillips, 2019). This negative net protein balance has actually been observed to be reached in as little as 6 hours following the onset of limb immobilization (Booth & Seider, 1979). Other pathways are also involved in the disbalance in muscle protein during immobilization, and the interaction is more complex than what is described above, but this falls outside the scope of this review (Bodine, 2013).

2.1.2 Physiological Consequences Following Limb Immobilization

Recent animal studies have also suggested physiological consequences to limb immobilization (Hu et al., 2017; Santos-Júnior et al., 2015). Indeed, a study on the immobilization of hind limbs in rats showed a significant increase in serum alkaline

phosphatase (which plays a role in bone density) and lactate dehydrogenase (a metabolic enzyme), as well as a decrease in blood calcium, total protein, and albumin (Santos-Júnior et al., 2015). While this evidence has not been reproduced in humans yet, it suggests that limb immobilization might affect the body as a whole and not just the limb affected. Hematology should thus be further investigated in future immobilization studies in humans in order to better understand the relationship between the muscular changes, the entire body, and what happens in the brain at the same time.

2.1.2 Neurological Consequences of Limb Immobilization

Central to this thesis, however, is the idea that a negative net muscle protein balance coupled with a loss of muscle mass and thickness are not enough to entirely explain the loss in strength during limb immobilization (Booth, 1982; Frontera & Ochala, 2015). Other factors, such as neurological factors, must thus play a role in the loss of contractile force during a period of disuse. For instance, one multiple regression analysis study showed that neural factors, principally central activation, were in fact responsible for a larger portion of the strength loss following immobilization than muscle loss itself (Clark, Manini, Bolanowski, & Ploutz-Snyder, 2006). When it comes to the neurological consequences of limb immobilization, there are two types of changes expected: changes in the peripheral nervous system (PNS) and changes related to the central nervous system (CNS).

2.1.2.1 Peripheral Neurological Consequences of Limb Immobilization

In the PNS, one potential mechanism for the loss in strength associated with limb immobilization is the disuse of neuromuscular junctions (NMJ). This idea arises from the fact that the relationship between the motor unit and its corresponding motor neuron, called the synapse, is extremely important in motor control and production of contractile force (Ferraro, Molinari, & Berghella, 2012). Despite the promising potential of this explanation, studies have not closely explored this avenue yet and researchers have not found a clear mechanism that

would support this hypothesis. It remains unclear how NMJ are involved in strength loss following immobilization with some studies suggesting that NMJ instability can occur during prolonged periods of bed rest (Bütikofer, Zurlinden, Bolliger, Kunz, & Sonderegger, 2011; Monti et al., 2021). Indeed, one study investigating 10-day bed rest observed an increase in C-terminal agrin fragment (Monti et al., 2021), an important biomarker associated with impairment in neuromuscular function following injuries (Sánchez-Castellano et al., 2020; Scherbakov et al., 2016) and that has been shown to precede NMJ degeneration in animal studies (Bütikofer et al., 2011). This remains the only study (Monti et al., 2021), however, to document an increase in C-terminal agrin fragment during immobilization, while one other study using even more extensive bed rest protocols failed to quantify such a change (Ganse, Bosutti, Drey, & Degens, 2021).

Muscle denervation is another mechanism related to the NMJ that may partly explain the loss in strength due to limb immobilization (Arentson-Lantz, English, Paddon-Jones, & Fry, 2016; Monti et al., 2021). Neural-cell adhesion molecule is a biomolecule responsible for the development of NMJ, contributing to the innervation with the muscles (Covault & Sanes, 1985). Its presence in adults indicates a novel attempt to connect the nerves to the muscles, suggesting that denervation has previously occurred (Covault & Sanes, 1985). It has been observed that during bed rest, levels of neural-cell adhesion molecule increase in the body in an attempt to maintain or re-establish NMJ (Arentson-Lantz et al., 2016; Monti et al., 2021). These changes, however, remain small and inconsistent between individual subjects (Arentson-Lantz et al., 2016; Monti et al., 2021).

2.1.2.2 Central Neurological Consequences of Limb Immobilization

The first central neurological factor to consider in strength loss, although it is more of an effect of the interaction between the CNS and PNS, is the capacity to voluntarily produce muscle contractions, a phenomenon called voluntary activation (VA). Indeed, after a

period of disuse, the ability to fully contract muscles is typically impaired, leaving out some unused contractile force (Clark, Mahato, Nakazawa, Law, & Thomas, 2014; Stevens et al., 2006). This VA can be quantified by supramaximal peripheral muscle stimulation or transcranial magnetic stimulation, recruiting additional – residual – contraction despite participants contracting at what they consider to be their maximum (Todd, Taylor, & Gandevia, 2004). Even if the consequence of a decreased VA, a reduction in maximal force production, is peripheral and results in a decreased contractile force, however, it is thought that this originates from the brain, supporting the importance of CNS-related changes in the consequences of immobilization (Post, Steens, Renken, Maurits, & Zijdwind, 2009).

Moreover, limb immobilization can also induce neuroplasticity such that changes in cortical thickness have been reported. For instance, one study found a significant thinning effect of the immobilized M1 and S1 following on average 16 days of upper-limb immobilization (Langer et al., 2012). These changes could potentially be a result of a decrease in arborization of the gray matter neurons, reduced glial cell volume, or decreased vasculature in the disused area (Langer et al., 2012). Another possible mechanism involving insulin-like growth factor 1 (IGF-1) has also been suggested in animal studies (Mysoet, Canu, Cieniewski-Bernard, Bastide, & Dupont, 2014; Mysoet, Dupont, Bastide, & Canu, 2015). Indeed, IGF-1 contributes to neurogenesis in motor learning and development and its concentration has been shown to decrease in the brain during muscle disuse. More specifically, IGF-1 concentrations underwent a significant decrease in the sensorimotor cortex as well as the striatum following hindlimb unloading of rats (Mysoet et al., 2014). A decrease in IGF-1 could thus be responsible, at least partly, for a decrease in neurogenesis rate and a loss of cortical thickness in humans as well.

On the other hand, immobilization can also lead to an increase in cortical thickness of the M1 associated with the non-immobilized limb, most likely caused by an increase in

unrestricted limb usage (Langer et al., 2012). A negative correlation between the increase in skill of the non-dominant non-immobilized limb and the change in cortical thickness has also been established (Langer et al., 2012). White matter is affected by immobilization as well, as a decrease in fractional anisotropy in the corticospinal tract associated with the immobilized limb has been demonstrated in arm immobilization (Langer et al., 2012). These results suggest that immobilization can lead to a disconnection of the disused brain regions from the rest of the body (Langer et al., 2012). Nevertheless, the literature regarding brain structural changes caused by immobilization is very limited, and the study by Langer et al. (2012) remains the only one to have shown these changes in healthy individuals. With structural changes seemingly occurring in the brain during immobilization, it is appropriate to ask if this neuroplasticity impacts functional mechanisms as well. It is well established that brain structure and function are closely related (Batista-García-Ramó & Fernández-Verdecia, 2018), promoting the need for studies investigating the functional impact of limb immobilization.

In a healthy brain, muscle contractions mainly arise from a signal originating in the M1, which is the direct output from the brain to the muscles (Taylor, Amann, Duchateau, Meeusen, & Rice, 2016). However, M1 is not the only region that participates in motor execution, which also involves other cortical areas such as the supplementary motor area (SMA), the premotor cortex (PMC), and the S1 (Yeom, Kim, & Chung, 2020). In addition to these cortical regions, the cerebellum also participates in the execution of motor tasks (Stoodley & Schmahmann, 2018), along with subcortical regions like the basal ganglia (which includes the substantia nigra and the striatum) and the thalamus (Groenewegen, 2003). Together, these regions integrate information and feedback from one another and form the desired motor output, during which they show elevated activation. In an immobilized limb, the important decrease in activation of these regions will make them subject to neuroplasticity, which induces changes in activation

and connectivity (Burianová et al., 2016; Garbarini et al., 2019; Lissek et al., 2009; Newbold et al., 2020).

These changes in brain function have been described multiple times, but with different fMRI paradigms, making it hard to truly reproduce results (Burianová et al., 2016; Farthing et al., 2011; Garbarini et al., 2019; Newbold et al., 2020). For instance, a study performed on three participants used daily fMRI scans to quantify changes in brain connectivity during and after upper-limb immobilization in the resting brain (Newbold et al., 2020). It was observed that the unused regions of the sensorimotor system (left M1 and S1, and right upper-limb representation of the cerebellum) became more strongly connected together, but disconnected from the rest of the sensorimotor network throughout the immobilization period (Newbold et al., 2020). These changes also correlated with synchronized spontaneous spikes of elevated activity in the unused brain regions at rest, suggesting a potential mechanism in which those spikes contribute to the segregation of the unused regions (Newbold et al., 2020). Considering that functional connectivity is usually stable on a day-to-day basis (Gratton et al., 2018), these changes support the fact that limb immobilization induces neuroplasticity that in turn affects the brain at rest. The present study, using a larger sample and a type of analysis based on previously described findings, was able to depict a more detailed picture of the different regions affected by limb immobilization.

The study by Newbold and colleagues (2020) remains the only one that studied the brain at rest during immobilization, but more information is available on task-based fMRI in situations of limb immobilization. One study on the effects of upper-limb immobilization used task-based fMRI scans at four different timepoints: timepoint 1 before immobilization, timepoint 2 immediately following immobilization of the left forearm, timepoint 3 after one week of immobilization, and timepoint 4 right after removal of the immobilizing cast (Garbarini et al., 2019). The task consisted in hand contractions (opening and closing the fist),

or to attempt a contraction when the limb was immobilized. The results showed an increased activation of the ventral premotor cortex (vPMC) when a participant's attempt to perform a contraction was prevented by the cast at timepoint 2, consistent with the vPMC's motor monitoring role (Garbarini et al., 2019). This increase went away at later stages of the immobilization (timepoint 3), suggesting that the brain "expected" the contraction to be prevented, again showing the occurrence of neuroplasticity. The fMRI results also showed a decrease in immobilized M1 activation following the immobilization and a stronger connectivity between the immobilized vPMC and S1 during contraction (Garbarini et al., 2019).

Another study also used an active fMRI paradigm to characterize changes in brain function following a 3-week wrist immobilization (Farthing et al., 2011). The fMRI, which was performed during a grip strength test before and after the immobilization, showed no decrease in activation of the disused M1 during contractions in contrast with the study of Garbarini and colleagues (2019) (Farthing et al., 2011). During participants' contraction of that same immobilized arm, there was also an increased activation in the contralateral posterior prefrontal cortex, as well as the ipsilateral ventral–occipital–parietal cortex and ventral inferior temporal gyrus (Farthing et al., 2011). In the case of the non-immobilized arm, there was increased activation of the vPMC and posterior portion of the lateral sulcus on the border of the inferior parietal and superior temporal cortices (Farthing et al., 2011). These changes are quite different than what was observed in the rest of the literature, but they still provide some insight on other brain regions that could undergo changes during limb immobilization.

While these three studies (Farthing et al., 2011; Garbarini et al., 2019; Newbold et al., 2020) all involved periods of immobilization of at least one week, a shorter period of disuse can also produce changes in brain activity (Burianová et al., 2016). Indeed, an upper-limb immobilization period as short as 24 hours showed a significant decrease in contralateral M1

activity at the end of the disuse period (Burianová et al., 2016). The researchers of this study recorded the brain activity during motor imagery of the constrained hand, and M1 activity was significantly lower after 24 hours of immobilization when compared to its activity during the same task before the constraint (Burianová et al., 2016).

The range of approaches used in the current literature regarding brain functional imaging provides insight on the different mechanisms involved in strength loss and other consequences of limb immobilization. It is clear from the studies presented that the primary sensorimotor regions (M1 and S1) are affected by immobilization. The vPMC and the cerebellum also seem to undergo changes in activation and connectivity during immobilization. Nevertheless, very little is known about the resting connectivity of these regions following immobilization. A better understanding of the changes in connectivity involving these regions could help point to the regions most impacted by limb immobilization.

2.1.3 Sex Differences in Limb Immobilization

Research suggests a clear difference between the outcomes found in men and women following restrictive limb immobilization. Although the loss in muscle mass incurred is similar for both men and women, female participants generally undergo a significantly larger loss of strength following muscle unloading caused by limb immobilization (Deschenes et al., 2009, 2012; Yasuda et al., 2005). This suggests a potentially larger involvement of the neurological factors explored when compared to the peripheral factors, which act directly on the muscles. It is important to note that one study has shown a higher strength loss magnitude in men than women (Miles, Heil, Larson, Conant, & Schneider, 2005) and that another study observed no difference in strength loss (Clark, Manini, Hoffman, & Russ, 2009). However, the former study used a far less restrictive protocol than actual joint immobilization called unilateral suspension and failed to quantify limb utilisation using accelerometers, making the results subject to individual differences in limb usage during the protocol. The latter study was underpowered,

as suggested by the individual participant results. Indeed, only ten participants (5 males, 5 females) took part in this study, and only one of the males lost as much strength as any of the five females, skewing the data for the male group. Therefore, it does seem like females tend to lose more strength than males following immobilization, despite a similar loss in muscle mass, suggesting more important changes in the neurological factors in females.

2.2 Functional Magnetic Resonance Imaging (fMRI)

fMRI is an imaging technique that allows to go further into brain function than the simple observation of anatomical landmarks. Instead, fMRI focuses on the operational mechanisms of the brain by reporting the blood-oxygen-level-dependent (BOLD) signal (Logothetis, 2008; Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). In other words, fMRI uses strong magnetic fields to measure the concentration of deoxyhaemoglobin in specific regions of the brain. A higher concentration of this deoxyhaemoglobin in a specific area suggests that oxygen has been used up by the activated brain regions (Logothetis, 2008; Logothetis et al., 2001). Although this technique has poor temporal resolution, it allows for a very high spatial resolution, because of which fMRI can provide extremely precise insights on the role and connections of different brain regions (Logothetis, 2008).

fMRI can be performed either as a task-based or resting-state imaging technique (Fox & Greicius, 2010; Greicius et al., 2009; Zhang et al., 2016). In task-based fMRI, the goal is to quantify the activation of specific regions during a given motor or cognitive task (Zhang et al., 2016). In resting-state fMRI, the goal is to observe the baseline functional activation of the different regions of the brain and to quantify the intrinsic segregation present between different networks (Logothetis, 2008; Zhang et al., 2016). Thus, while task-based fMRI can be useful to better understand specific mechanisms of the brain, resting-state fMRI provides a more holistic approach to visualize connections in the brain. Paired with an intervention such as limb immobilization, resting-state fMRI can truly help grasp what changes occur following such a

restrictive protocol. Ultimately, the insight gained from this technique could allow the prevention of the undesired effects of this therapeutic technique.

2.2.1 Resting-State Functional-Connectivity (rs-FC)

Assessing activation of certain brain regions at rest provides more insight when performed during a task. At rest, rs-FC tends to be more useful, depicting a more complete picture of brain mechanisms using the timing of spontaneous discharge of different brain regions (Biswal, Yetkin, Haughton, & Hyde, 1995; Fox & Greicius, 2010; Greicius et al., 2009; Seitzman, Snyder, Leuthardt, & Shimony, 2019). These spontaneous changes in discharge do not represent random fluctuations in brain activity (Fox & Raichle, 2007). Rather, it has been shown that they are closely related to known anatomical systems and that they cannot be attributed to respiratory or cardiovascular factors (Fox & Raichle, 2007). The fluctuations occurring at rest, instead, most likely represent the Default Mode Network (DMN), which is a brain network representing the stay ready state of the brain for effective use whenever needed (Seitzman et al., 2019; Thompson & Varela, 2001). Further supporting the importance of resting, baseline brain activity is the fact that only about 5% of the total brain energy consumption can be attributed to changes in brain activity (Raichle, 2015). Thus, by using regression analyses, rs-FC is able to look at low frequency (0.01-0.08 Hz) fluctuations and establish functional relationship between regions of the brain that tend to activate at the same time (Fox & Greicius, 2010; Greicius et al., 2009; Seitzman et al., 2019).

In terms of functional connections, it is expected that brain regions with a direct communication via bundles of axons will be strongly connected functionally as well (Fox & Greicius, 2010). However, even regions with no explicit structural relationship – regions that lack a monosynaptic connection – can be functionally connected, creating different networks such as the visual and somatomotor networks (Seitzman et al., 2019). These regions are thus thought to form indirect connections via other regions of the brain. By allowing the

quantification of functional connections, rs-FC therefore allows to better understand the mechanisms at work without being limited by anatomical and structural landmarks.

Connectivity between regions at rest can even help predict the connectivity of certain regions during given tasks. For instance, the lateralization of the left sensorimotor system at rest could predict the lateralization of that same region during a task performed with the right hand (Fox & Raichle, 2007). These results suggest that the baseline activity in motor regions during period of immobilization is most likely present in order to prepare the motor network to react quickly and effectively when necessary.

2.2.1.1 Different Analysis Techniques in rs-FC

While all rs-FC relies on the same principles of spontaneous activation of different regions in the brain at rest, there are different ways to set up the analysis depending on the exact purpose of the study. Techniques such as frequency domain analysis, regional homogeneity, and graph theory can all be used in the process of analyzing results obtained in rs-FC fMRI. However, the two most widely used techniques are called independent component analysis (ICA) and seed-based analysis (Cole, Smith, & Beckmann, 2010; Smitha et al., 2017).

ICA is an analysis technique that performs a whole brain comparison of functional connectivity, looking at multiple different voxels to identify synchronization in activation (Smitha et al., 2017). ICA is a data-driven technique that provides a complete overview of the resting networks in the brain. It is most commonly used in group-level comparisons (Smitha et al., 2017). Nevertheless, ICA has low signal-to-noise ratio, and previous expertise is necessary to distinguish the relevant signal from the noise, on top of which interpreting the results is not as straightforward as with seed-based analysis (Smitha et al., 2017).

Seed-based analysis consists in *a priori* choosing a set of regions of interest (ROIs) based on the literature and to extract the time series for this specific regions called a seed (Cole et al., 2010). The extracted data are then used as a regressor in a linear correlation analysis with

time series from the rest of the brain to establish functional activation relationships between the ROI and other regions. The primary advantage of seed-based analysis is its hypothesis-driven nature. In other words, this strategy offers a direct answer to a specific question and allows to define connections between specific regions of the brain. The results provide a straightforward explanation to what is happening, and seed-based analysis has been shown to have moderate to high test-retest reliability (Cole et al., 2010). Nevertheless, this type of analysis remains subject to bias in the seed selection process, and can also prevent from getting an absolutely complete picture of the complexity of brain networks (Cole et al., 2010).

Chapter 3: Manuscript

Changes in Brain Resting-State Functional Connectivity Following a 2-Week Upper Limb Immobilization in Female Participants.

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3.1 Abstract

Limb immobilization is known to cause significant losses in muscle strength and mass as early as three days following immobilization onset. However, the loss in strength tends to be larger than the loss in muscle mass, suggesting that factors other than muscle atrophy contribute to the decrease in force production. Among those factors, it is thought that limb immobilization can induce neuroplastic changes which can then impact muscle strength. Little is known on those brain-related changes, although the sensorimotor system seems to be the most affected. Functional Magnetic Resonance Imaging is a technique that can measure resting-state functional connectivity, a measure of the synchronicity of the spontaneous discharge of different brain regions at baseline. The present study thus aimed at determining whether brain resting-state functional connectivity is affected following a 2-week elbow immobilization period. Twelve female participants were scanned before and after completing the immobilization period. A seed-based analysis was performed using seeds associated with cortical, subcortical, and cerebellar sensorimotor regions of the brain. The results showed changes involving predominantly cerebellar connectivity. The immobilization period caused a decrease in connectivity between the motor cerebellar region of the immobilized arm and the left temporal lobe, and an increase between the same cerebellar region and the supplementary motor area. The seed placed in a premotor area associated with the non-immobilized arm and the motor cerebellar region of the same arm increased in connectivity following the immobilization. The region of the motor thalamus associated with control of the immobilized arm increased its connectivity with the immobilized posterior insula. Overall, changes in connectivity occurred in regions typically associated with error detection and motor learning, suggesting a potential early reorganization of the spontaneous brain discharge as early as within two weeks of elbow immobilization, causing a loss in error detection capacity in the immobilized arm and an increase in error detection capacity in the non-immobilized arm.

3.2 Introduction

It is well established that limb immobilization leads to a significant decrease in muscle contractile force and a loss in muscle mass (Booth, 1982; Marusic et al., 2021) as early as seventy-two hours after the beginning of immobilization (Gaffney et al., 2021). While the consequences of immobilization at the level of the muscle are well understood (Booth, 1987) there is abundant evidence showing that the muscle atrophy caused by limb immobilization does not tell the whole story when it comes to the loss in muscle strength (Booth, 1982; Campbell et al., 2019), suggesting that neural factors must contribute to the loss in force production. This phenomenon is especially impactful in women who generally lose more strength following limb immobilization despite a similar loss in muscle mass as men (Deschenes et al., 2009, 2012; Yasuda et al., 2005). These neural factors include, but are not limited to, muscle denervation as well as and brain- and spinal-related changes (Marusic et al., 2021). Although the effects of the neural changes resulting from limb immobilization have been demonstrated, the underlying mechanisms are not fully understood despite their crucial importance (Campbell et al., 2019). A better understanding of those neural factors would help characterize the brain mechanisms involved in immobilization and potentially contribute to developing strategies to mitigate the negative effects of limb immobilization to prevent the associated loss in strength and coordination experienced by individuals undergoing immobilization periods (Campbell et al., 2019; De Marco et al., 2021; Moisello et al., 2008).

Magnetic resonance imaging (MRI) has been used to better understand immobilization-induced changes in supraspinal substrates. Structural changes, such as cortical thinning and thickening and changes in white matter structure, following immobilization have been quantified using anatomical MRI sequences (Langer et al., 2012; Sterr et al., 2013). While these studies provide important information regarding structure, functional changes would be expected to precede structural changes. Functional MRI (fMRI) scans have allowed for the

assessment of the functional consequences of complete immobilization of a limb on the brain (Burianová et al., 2016; Farthing et al., 2011; Garbarini et al., 2019; Lissek et al., 2009; Newbold et al., 2020).

Only four studies have assessed brain activation in limb immobilization. Despite a very limited pool of research, the current literature provides somewhat consistent results, suggesting a deactivation of the sensorimotor network, which is the most affected brain network in limb immobilization (Burianová et al., 2016; Garbarini et al., 2019; Lissek et al., 2009). Indeed, both Burianová et al. (2016) and Garbarini et al. (2019) observed a reduction in primary motor cortex (M1) activation following immobilization, while Lissek et al. (2009) observed similar results in the primary somatosensory cortex (S1). Nevertheless, one study did fail to observe changes in activation of the disused M1 of participants following a 3-week left arm immobilization (Farthing et al., 2011), although changes in activation were observed in regions associated with more cognitive functions.

Only one study has quantified the effect of immobilization on resting state functional connectivity (rs-FC) (Newbold et al., 2020). In contrast to task-based fMRI, resting-state fMRI provides insight on the functional connectivity between different brain regions at rest (Fox & Greicius, 2010; Greicius et al., 2009; Newbold et al., 2020). rs-FC and task-based fMRI are closely related, as rs-FC can predict which brain regions is to be activated during a task (Fox & Raichle, 2007). Newbold and colleagues (2020) used a whole brain rs-FC analysis and showed a segregation of the disused sensorimotor network, including the contralateral M1 and S1, and the ipsilateral upper-limb representation of the cerebellum. The regions of the disused network became more strongly connected together but disconnected from the rest of the sensorimotor brain network. Garbarini et al. (2019) used fMRI to measure connectivity during hand contraction and found that, at any point during immobilization, the ventral premotor

cortex contralateral to the immobilized arm was more strongly connected to the S1 of the same hemisphere, further suggesting the occurrence of segregation.

While these studies provide some insight on the impact of immobilization on the sensorimotor network, the mechanisms, and the extent to which this occurs remain unclear because of the heterogeneity of the active fMRI protocols used in previous studies. Resting fMRI provides insight on the brain at rest, producing results that are applicable and generalizable to different tasks, but only three participants have undergone an immobilization protocol using this technique, limiting the applicability of the results. The aim of this study was thus to quantify the changes in rs-FC of the sensorimotor network that occur after a 14-day upper-limb immobilization period in female participants, who are more affected by limb immobilization. Although it is still early to conclude on the exact consequences of limb immobilization on brain connectivity, these studies suggest that immobilization does have an impact that extends beyond the changes occurring in muscle physiology, structure, mass, and strength, altering supraspinal circuitry associated with sensorimotor function. Based on the previous literature, a segregation of the disused sensorimotor network was expected (Garbarini et al., 2019; Newbold et al., 2020). Such a segregation has been characterized by higher connectivity between disused regions (i.e., contralateral M1 and S1, and ipsilateral cerebellum) and lower connectivity with the rest of the sensorimotor network (Garbarini et al., 2019; Newbold et al., 2020). It was also expected that changes in connectivity with the M1 would occur because of its involvement in motor output and because its activation is typically reduced following immobilization (Burianová et al., 2016; Garbarini et al., 2019; Lissek et al., 2009).

3.3 Materials and Methods

3.3.1 Participants

Twelve right-hand dominant female participants (aged 21 years $\pm$ 2 years) enrolled in the current study. Participants had a mean body mass index of 21.5 kg/m² ($\pm$ 3.2 kg/m²) and

reported a regular menstrual cycle. Participants were healthy as characterized by basic health measurements (heart rate, blood pressure, body composition measurement) and a health questionnaire, with no history of smoking, pregnancy, history of brain trauma, neurological disease, movement disease, mental disease, peripheral nerve damage, use of medications or supplements known to affect protein metabolism (e.g., corticosteroids, non-steroidal anti-inflammatory drugs, prescription strength acne medications, creatine, fish oil) or any MRI or transcranial magnetic stimulation (TMS) contraindication such as metal implants or non-removeable medical devices (Rossi, Hallett, Rossini, & Pascual-Leone, 2009).

This study was approved by the McGill University Faculty of Medicine Institutional Review Board and followed the guidelines of the Helsinki Declaration of 1975 (revised October 2013). All participants were informed of the nature of the study, procedures, and potential risks before providing informed written consent.

3.3.2 Study Design

The rs-FC assessment was part of a larger study (Seo, et al., 2022, *in preparation*) including the measurement of various other outcomes (e.g., muscle strength, voluntary activation, cortical excitability) before and after a 14-day left (non-dominant) elbow immobilization. During the pre-immobilization visit, participants underwent a brain T1 anatomical MRI and resting-state fMRI, a session of TMS to localize the M1 representation of the biceps brachii in both brain hemispheres and measure cortical excitability, and measurements of other clinical outcomes further described in the Data Acquisition section (3.3.4). This session was immediately followed by a 14-day elbow immobilization period, after which the same measurements were obtained. Scans were performed on the same day prior to installing the brace and minutes following the end of the immobilization period, respectively.

3.3.3 Limb Immobilization

The elbow immobilization at 90 degrees of flexion was achieved with a metal brace (Donjoy, Lewisville, USA) fixed in place and tied with single-use zip ties that were labeled to ensure that any tampering with the ties or removal of the brace would be easily detected. During the immobilization period, participants were allowed to use the hand of the immobilized arm to perform tasks of daily living, but the brace fully prevented any movement of the elbow joint. Participants were instructed to refrain from moderate to intense physical activity, alcohol, and cannabis from 48 hours before the immobilization period to the end of the study, and to avoid caffeine at least 24 hours before each experimental visit.

3.3.4 Data Acquisition

The pre- and post-immobilization MRI images were obtained using a Siemens 3T Trio Scanner (Siemens, Knoxville, TN) at the Montreal Neurological Institute (MNI) in Montreal, Canada. The protocols for brain imaging for each participant consisted in a T1-weighted anatomical MRI (acquisition time = 6:44, 224 slices, voxel size = 0.8 mm³ isotropic, echo spacing = 7.7 ms, flip angle = 9 deg) and a BOLD MOSAIC resting fMRI (acquisition time = 7:07, 700 volumes, voxel size = 3 mm³ isotropic, echo spacing = 0.54 ms, 48 slices, flip angle = 50 deg). During the resting-state fMRI scan, participants were asked to lie still, stay awake, not think about anything, and to fixate a cross in front of them.

As part of the larger study, the M1 representations for each biceps brachii muscle (biceps brachii hotspot) was localized with TMS using a 60-mm dome coil attached to a Magstim 200² stimulator (Magstim Company, UK) and a neuronavigation system (Brainsight frameless stereotaxic neuronavigation system; Rogue Research Inc, Montreal, Canada). During the TMS protocol, muscle activity was measured using monopolar surface electromyography (sEMG; Biopac MP150 acquisition system, sampled at 10kHz 5kHz on a 16-bit analog-to-digital board, amplified and bandpass filtered; 10-5000Hz; Biopac Systems, Inc.) with pre-

geled electrodes (Biopac Systems, Inc.) placed in a belly-tendon montage (one electrode over the biceps muscle belly and one electrode on the distal biceps tendon) with the ground electrode placed on the styloid process of the ulna. The cortical hotspots of the immobilized and non-immobilized biceps brachii were identified as the cortical positions eliciting 2 consecutive motor evoked potential responses in the relaxed muscle with greater amplitude than the surrounding area.

As part of a larger study (Seo et al., 2022, *in preparation*), muscle strength and voluntary activation were also measured before and after the immobilization period. Muscle strength was measured using isometric and isokinetic maximal voluntary contractions in arm flexion and extension before and after the immobilization period using an isokinetic dynamometer (Biodex 4 ProTM, Biodex medical instruments, Shirley, USA). Voluntary activation was measured using peripheral muscle stimulation (Digitimer, Welwyn Garden City, Herfordshire, UK) to perform the doublet twitch interpolation technique. One stimulation was delivered at peak torque and the second one three to five seconds after the contraction, and participants were verbally encouraged. Torque data for voluntary activation was recorded using the same isokinetic dynamometer as for muscle strength measurements.

3.3.5 Data Analysis

For neuro-imaging preprocessing, analysis, and statistical computations, a resting-state pipeline to analyze data relying on FSL 5.0.8 (FMRIB Software Library, Oxford, UK) and MATLAB 2018b software (<http://www.mathworks.com>) developed by the Center for Research on Brain, Language and Music (www.crblm.ca) was used.

3.3.5.1 Preprocessing

Preprocessing steps followed the protocol used previously (Potvin-Desrochers, Atri, Martinez Moreno, & Paquette, 2022; Potvin-Desrochers, Mitchell, Gisiger, & Paquette, 2019). Specifically, for each individual scan; the first 5 images of the timeseries were removed,

ensuring that the signal had time to stabilize; the linear registration transformations were calculated to coregister the resting-state, the anatomical and MNI 152 spaces; slice-timing correction using Fourier-spacetime-series phase shifting was performed; the brain was extracted from T1 anatomical scans using FSL's Brain Extraction Tool (BET); motion correction was performed using FSL's Linear Image Registration Tool (FLIRT) using rigid-body transformations (3 rotations and 3 translations); global intensity was normalized using FSL FMRIB Expert Analysis Tool (FEAT); spatial smoothing was performed using a Gaussian kernel of FWHM 6 mm; a band-pass butterworth filter in MATLAB (filtfilt in Matlab's signal processing toolbox) was used to preserve frequencies in the 0.01–0.1 Hz range; and motion outlier measurements were removed (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012).

3.3.5.2 Seeds

FC was measured using a seed-to-voxel analysis, temporally correlating the spontaneous fluctuations in the blood-oxygen-level-dependent signal (BOLD) of regions of interest (ROI) with the rest of the voxels in the brain. Seeds were created using masks in Montreal Neurological Institute (MNI) space to represent the sensorimotor network-specific ROIs potentially involved in upper-limb immobilization. The cortical seeds consisted of the upper-limb representations of M1, S1, along with the premotor cortex (PMC) and the supplementary motor area (SMA). The subcortical seeds were different lobules of the cerebellum, thalamus, putamen, and globus pallidus.

The seeds for the immobilized and non-immobilized M1 representations were centered at the MNI coordinates of the biceps muscle hotspot as determined individually for each participant following the TMS hotspot localization protocol described in the data acquisition section (3.3.4). The M1 seeds consisted of four-mm-radii spheres. All the remaining seeds were positioned according to the literature and consisted of six-mm-radius spheres to account for variability in cortical organization across participants. Specular bilateral seeds were produced

using opposite x coordinates and keeping y and z values (Garbarini et al., 2019). The immobilized and non-immobilized S1 representations of the upper arm seeds were created using Roux, Djidjeli, and Durand's (2018) mapping of the somatosensory cortex [x: +/-27.7 y: -34.5 z: 70.35] (Roux, Djidjeli, & Durand, 2018). Garbarini et al. (2019) also suggested the involvement of three other cortical areas: vPMC [x = +/-56 y = +6 z = +30], dorsal PMC [x = +/-4 y = -6 z = +58], and SMA [x = +/-26 y = -10 z = +62]. The cerebellar lobules involved in upper limb control were determined by Mottolese et al. (2013) and matched to MNI coordinates proposed by Bernard et al. (2012) (lobules VI [x: +/-36 y: -48 z: -43], VIIb [x: +/-32 y: -72 z: -51], VIIa [x: +/-32 y: -56 z: -55], VIIb [x: +/-18 y: -40 z: -54], and IX [x: +/-11 y: -53 z: -41]) and were also six mm-radius spheres (Bernard et al., 2012; Mottolese et al., 2013). The globus pallidus and putamen masks were created using the Basal Ganglia Human Area Template atlas (Prodoehl, Yu, Little, Abraham, & Vaillancourt, 2008). The thalamus masks were created with the WFU PickAtlas tool (Maldjian, Laurienti, Kraft, & Burdette, 2003) using Statistical Parametric Mapping software version 12 (SPM12, Wellcome Centre for Human Imaging, London, UK). A total of 36 seeds (18 in each hemisphere) were used for analysis.

3.3.5.3 Functional Connectivity Analysis

The protocol used by Potvin-Desrochers et al. (2019, 2022) was followed to obtain the rs-FC results. Accordingly, the individual FC maps were obtained using a rs-FC regression analysis in native space. Before performing the regression, the time series of the seeds were obtained using the steps detailed in the preprocessing section (3.3.5.1), after which the BOLD signal from all voxels within each seed was averaged to calculate the mean time series. Moreover, the time series of the nuisance variables were used as predictors in a general linear model with FEAT. The nuisance variables included in the analysis were: cerebrospinal fluid (CSF), white matter (WM), global signal, motion outlier volume masks, as well as the motion

parameters obtained in the motion correction step (translation and rotation about the x, y, and z axes). More specifically related to the nuisance caused by CSF and WM, the physiological noise originating from the CSF and WM was eliminated by removing masks for the two types of tissue from the T1 anatomical image with segmentation using an 80% tissue type probability threshold. The BOLD signal from CSF and WM was then averaged to produce a mean signal that could then be used as a nuisance factor.

In order to perform pre- and post-immobilization group comparison, each individual FC map in native space was linearly transformed to MNI standard space using FLIRT. To do so, results from the pre- and post-immobilization fMRI were first aligned to native space from their respective T1 scans using a seven degree-of-freedom transformation. The T1 images were subsequently matched to MNI 1 mm³ standard space using a 12 degrees-of-freedom linear affine transformation. rs-FC maps were transformed in MNI 152 standard space using the transformation matrices.

3.3.5.4 Statistical Analysis

Pre- and post-immobilization comparisons were performed using two mixed-effect models using a Bayesian modeling scheme in FLAME, FSL (Woolrich, Behrens, Beckmann, Jenkinson, & Smith, 2004). Following the method used in Potvin-Desrochers et al. (2019, 2022), correction for multiple comparisons was performed using a Gaussian random field theory, using a cluster threshold of $Z > 3.1$, and a cluster significance of $p < 0.05$ (Worsley, 2001). A secondary analysis was also performed using a less conservative threshold of $Z > 2.6$ to reveal regions potentially undergoing changes in connectivity. The specific brain region represented by each resulting cluster was identified using the WFU PickAtlas tool in SPM12 (<http://www.fil.ion.ucl.ac.uk/spm/>). The cortical regions contralateral and the subcortical regions ipsilateral to the immobilized arm were considered disused or immobilized regions, whereas the other hemisphere was non-immobilized.

3.4 Results

Between-group comparisons, presented in Table 1 and Figure 1, show the regions with altered rs-FC following immobilization. Overall, rs-FC with the cerebellum is most frequently altered after immobilization with significant changes with three different regions. Specifically, there was an increase in rs-FC between the disused cerebellar lobule VIIIa and the bilateral SMA after two weeks of immobilization. Post-immobilization, there was a positive rs-FC between these regions, although they were not functionally connected pre-immobilization (Figure 2). A cerebellar region of the non-immobilized lobule VIIb also had a change in rs-FC with the seed placed in the left vPMC, associated with the non-immobilized arm, going from a negative to a positive rs-FC. A similar pattern reversal was also observed in the immobilized ventrolateral nucleus (VL) of the thalamus and the immobilized posterior insula, which went from a negative rs-FC pre-immobilization to a positive rs-FC post-immobilization. Finally, the disused lobule VIIIa significantly decreased its rs-FC with the left middle temporal gyrus (MTG), a region that is not as functionally lateralized as the rest of the regions discussed here. The lobule VIIIa and the MTG went from a positive rs-FC before the immobilization to no rs-FC after the immobilization.

Along with these changes in rs-FC, the 14-day immobilization period also led to a decrease in isometric muscle strength in flexion and extension of the immobilized elbow, a decrease in excitability in the non-immobilized M1, and an increase in excitability in the immobilized M1. Correlations with muscle immobilization outcomes (torque production and voluntary activation) were measured, but only one significant correlation was observed after correction for multiple comparisons (see Supplementary Material). The rs-FC between the non-immobilized vPMC and cerebellum positively correlated to the change in immobilized arm isometric flexion after the immobilization ($r = .770$, $p = 0.003$).

The secondary analysis with a less stringent threshold at $Z > 2.6$ revealed that 15 additional clusters were altered by the 14-day immobilization period (Supplementary Material). These changes included an increase in rs-FC between: the immobilized M1 and the posterior cingulate cortex of the immobilized hemisphere, the immobilized S1 and the superior temporal gyrus of the immobilized hemisphere, as well as the immobilized ventral anterior nucleus of the thalamus and immobilized insula.

Table 1. Clusters Representing Significant Changes in Functional Connectivity

Contrasts	Clusters								<i>p</i>
	Seeds	Regions	Brodmann Areas	Size (voxels)	MNI Coordinates			Max Z-value	
					X	Y	Z		
Pre > Post	Left Cerebellum Lobule VIIIa	Left Middle Temporal Gyrus	37	1522	-54	-69	6	4.52	0.002
	Left Cerebellum Lobule VIIIa	Bilateral SMA	6	1145	1	1	63	4.19	0.010
Pre < Post	Left vPMC	Right Cerebellum Lobule VIIb	N/A	1055	8	-76	-38	4.52	0.036
	Right VL	Right Posterior Insula	13	997	40	-4	15	5.05	0.030

MNI: Montreal Neurological Institute, SMA: Supplementary Motor Area, vPMC: ventral premotor cortex, VL: Ventrolateral Nucleus of the thalamus

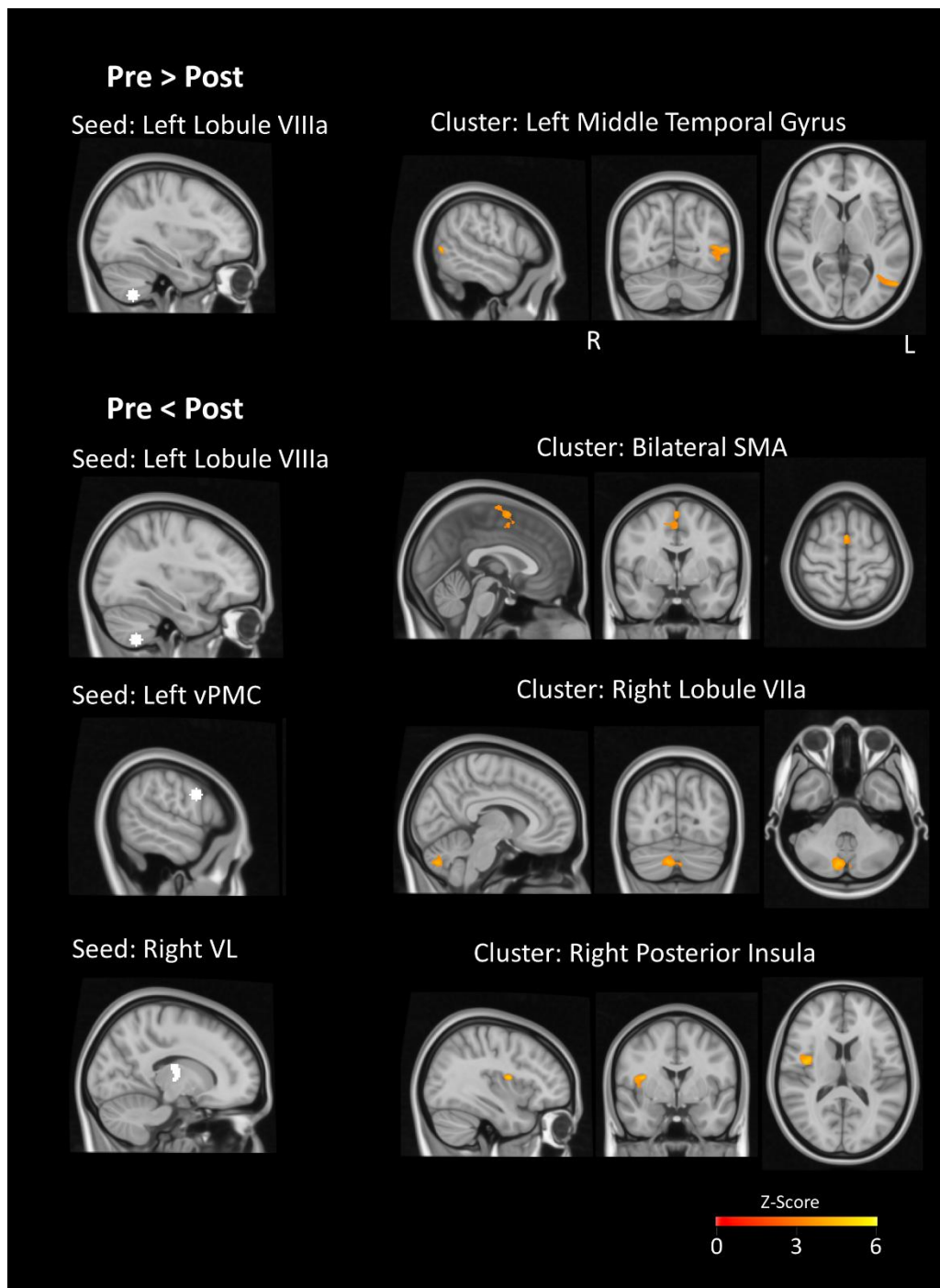


Figure 1. Statistical maps (Z-score) showing all significant changes in rs-FC between the seed and the highlighted cluster after the immobilization period. Pre > Post shows an increased rs-FC between the seed (left) and rs-FC map (right), while Pre < Post indicates a decrease in rs-FC following immobilization. The level of significance was set at $P < 0.05$, corrected. R: right, L: left, SMA: supplementary motor area, vPMC: ventral premotor cortex, VL: ventrolateral nucleus of the thalamus, rs-FC: resting state functional connectivity.

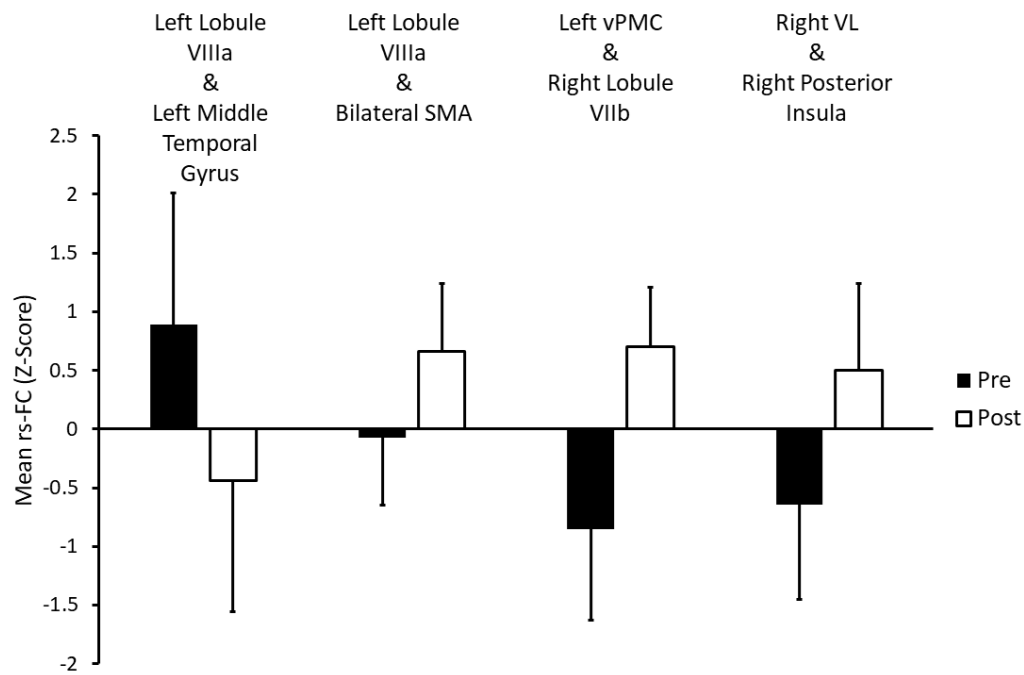


Figure 2. Mean rs-FC (Z-Score) between each pair of seed and its significant cluster before (pre; in black) and after (post; in white) the 2-week limb immobilization. Each comparison represents a significant difference as determined by the higher-level rs-FC analysis described in the methods. Positive mean values represent a positive rs-FC while negative mean values suggest a negative rs-FC, also known as anti-coupling. rs-FC: resting state functional connectivity, SMA: supplementary motor area, vPMC: ventral Premotor Cortex, VL: ventrolateral nucleus of the thalamus.

3.5 Discussion

The purpose of the present study was to quantify the changes in rs-FC within the sensorimotor network in healthy female participants over a fourteen-day immobilization period. The results were expected to show a segregation of the disused sensorimotor network along with changes in connectivity between the M1 and other regions of the sensorimotor network. However, as opposed to Newbold et al. (2020), no clear segregation was observed following immobilization in the present study. Instead, we observed that the immobilization

period mainly impacted on the cerebellum's connectivity, a key brain region for error detection, motor learning and skill acquisition (Popa, Streng, Hewitt, & Ebner, 2016). On the other hand, connectivity involving the M1 was only modestly increased with the right posterior cingulate cortex. Overall, our 14-day elbow immobilization led to changes predominantly involving regions associated with movement coordination, error detection, and motor learning (e.g., cerebellum and vPMC) much more so than regions related to motor drive and the direct communication between the brain and the muscles (e.g., M1 and S1).

Cerebellar connectivity underwent the most important changes following the 14-day elbow immobilization. Decreased connectivity was observed between the seed positioned in the immobilized cerebellar lobule VIIla, a region involved in motor control of the upper limbs (Guell & Schmahmann, 2020; Mottolese et al., 2012), and the left MTG, a very complex regions with functions encompassing a variety of different networks including the sensorimotor network (Ardila, Bernal, & Rosselli, 2015) and involved in action-feedback monitoring (van Kemenade et al., 2019). Specifically, the two regions went from a positive connectivity to no connectivity after immobilization. The lack of connectivity post-immobilization suggests less communication and cooperation between the cerebellar seed and the left MTG. In fact, during occurrences of error detection, results have shown an immediate increase in connectivity between bilateral MTG and the cerebellum ipsilateral to the side of the action (van Kemenade et al., 2019). This increase in connectivity most likely results from the MTG transmitting sensory mismatch information to the cerebellum, which in turn contributes to motor learning (van Kemenade et al., 2019). The loss in connectivity observed at rest here suggests that the disuse of the arm induced a decrease in the readiness of the MTG to communicate relevant information regarding errors occurring during movement. If this interpretation is accurate, it could explain why limb immobilization has been shown to decrease the capacity to perform complex motor tasks (De Marco et al., 2021; Moisello et al., 2008).

In contrast to its loss in connectivity with the left MTG, this same cerebellar seed located in the disused lobule VIIIa had an increase in connectivity with bilateral SMA. Although it has been shown that the SMA and the cerebellum co-activate during motor tasks in healthy people (Narayana et al., 2012), there was no such connectivity between the two regions pre-immobilization in this study, suggesting that the SMA and the cerebellum do not tend to be connected at rest in our sample. The SMA is a region involved in movement generation and control (Akkal, Dum, & Strick, 2007; Nachev, Kennard, & Husain, 2008), motor planning (Grèzes & Decety, 2002) and temporal organization of sequential movements (Tanji & Shima, 1994), along with participating to the direct motor output (Luppino, Matelli, Camarda, & Rizzolatti, 1993). Therefore, the positive post-immobilization connectivity could represent an increased need for the immobilized cerebellum to communicate error messages to the SMA to indicate that the movements being planned are not actually being produced by the immobilized arm. Along with the loss in connectivity between the MTG in the non-immobilized hemisphere and the cerebellar seed, the increased connectivity with the SMA at rest could represent a shift in the allocation of resources in the cerebellum. After two weeks of elbow immobilization, the proprioceptive signal arising from the immobilized arm and the error signals transmitted by the MTG remain constant, and detection of errors is not a priority anymore. This shift might have reduced the need for communication with the error detection region (left MTG) and increased the need for a change in the motor planning regions (the cerebellum and the SMA), forcing the cerebellum and the SMA to be in a “ready” state for communication. The reduced communication between the left MTG and the cerebellum might thus reduce the ability to detect new, unexpected errors, likely affecting coordination and motor skills performance. Studies have shown that coordination and motor skill deficits following immobilization are observed (De Marco et al., 2021; Moisello et al., 2008). Our findings suggest that changes involving connectivity of the cerebellum and the MTG at rest is a potential

mechanism responsible for this behavioral change, and that the increased connectivity with the SMA is an attempt to correct those motor programs causing errors.

Connectivity changes in the cerebellum were not limited to the immobilized side, but also extended to the non-immobilized cerebellar hemisphere. The significant change from a negative to a positive connectivity between the non-immobilized cerebellar lobule VIIb, a region associated with hand and arm movement, and the seed placed in the immobilized vPMC suggests a change in the synchronization pattern between the two regions and an increase in cooperation. Negative connectivity (i.e., anti-coupling) indicates that when spontaneous discharge occurs in one region, the other one decreases its activity synchronously. Thus, the two regions activated in an organized but opposite direction before immobilization showing involvement in similar networks. Post-immobilization, however, the two regions had a positive connectivity, which could suggest that the immobilization resulted in a more direct communication between the two regions, similarly to the activation patterns between the MTG and the cerebellum during the transmission of error signals. Similarly to the MTG, the vPMC tends to activate when there is a mismatch between movement planning and movement execution, supporting the role of the vPMC in error detection and performance monitoring (Acuña, Pardo-Vázquez, & Leborán, 2010; Garbarini et al., 2019; Pardo-Vazquez, Leboran, & Acuña, 2008, 2009). As opposed to the loss in connectivity between the left MTG and immobilized cerebellar seed described above, the shift in connectivity patterns between the non-immobilized vPMC and cerebellar lobule VIIb potentially suggests an increase in communication. This change could have been caused by the overuse of the non-immobilized arm, and has the potential to augment the capacity to detect and integrate errors in the non-immobilized limb. This is also the rationale behind constraint-induced therapy, a strategy used to improve motor coordination in, among other populations, people who have suffered from a stroke (Corbetta, Sirtori, Castellini, Moja, & Gatti, 2016).

Finally, only one of the four significant changes in rs-FC did not involve the cerebellum. The immobilized VL nucleus of the motor thalamus and the immobilized posterior insula underwent a shift from a negative connectivity to a positive one following the immobilization period, similarly to the connectivity between the vPMC and the cerebellar region previously described. The VL relays motor information from the globus pallidus internal, cerebellum, and premotor cortex to associative regions of the cerebral cortex as well as the SMA and PMC (Bosch-Bouju, Hyland, & Parr-Brownlie, 2013; Herrero, Barcia, & Navarro, 2002). It also plays a role in motor learning, as lesions to that structure alter skill acquisition (Canavan et al., 1989). On the other hand, the posterior insula is one of the least understood regions of the brain (Uddin, Nomi, Hébert-Seropian, Ghaziri, & Boucher, 2017). It is involved in somatosensory processing (Uddin et al., 2017), intentional movement and body awareness (touch, proprioception, kinesthesia) (Tinaz et al., 2018), as well as body orientation, environmental monitoring, and response selection (Cauda et al., 2011). It is also partly responsible for initiation, evaluation and modification of movements (Brass & Haggard, 2010; Paulus et al., 2009). Because of the variety of roles in which the insula is involved, it is difficult to pinpoint exactly what this increase in connectivity means, especially since these two regions have been shown to typically have a positive connectivity at rest (Cauda et al., 2011). Nevertheless, both the VL and the posterior insula seem to be important for motor learning and error detection, which are recurring functions across all four significant changes in rs-FC observed in the present study. The passage from an anti-coupling relationship to a positive rs-FC could potentially indicate an increase in the number of efferent copies sent directly from the VL to the insula for it to perform the necessary adjustments in the motor command for the outcome to be closer to what was intended. This relationship would be analogous to the one between the cerebellar seed and the SMA, which also underwent an increase in connectivity.

Only one change in connectivity (between the left vPMC and right cerebellar lobule VIIb) observed in the present study was correlated with a change in strength (immobilized arm flexion), while none of the changes correlated to voluntary activation and cortical excitability. This correlation, is also counterintuitive, since it shows a significant positive relationship between the change in strength in the immobilized left arm and rs-FC changes involving regions associated with the right-arm, which could be thought to be unrelated. Thus, more investigation is needed to truly understand the meaning of this correlation. The lack of other significant correlations further shows that our findings seem to be more closely related to motor learning and error detection than to brain regions involved in direct motor output and muscle drive. Indeed, the changes in connectivity related to the immobilized arm suggested a reduced ability to detect errors and an attempt by the brain to modify motor planning in response to the immobilization. During the immobilization, the demand imposed on the non-immobilized arm was most likely increased, which enhanced the need for error detection and motor learning on this side. This is important to take into account when considering the effects of immobilization on coordination and motor skill performance (De Marco et al., 2021; Moisello et al., 2008). Future studies should attempt to correlate connectivity changes such as the ones presented here to behavioral changes in motor skill performance. Ultimately, a better characterization of the effects of immobilization on both coordination and error detection-related changes in the brain could promote the need for regaining motor coordination following immobilization. This could even have an eventual impact on rehabilitation programs. These programs are often focused on strength recovery, which remains crucial, but the results of the present study suggest that immobilization could have a profound impact on neural mechanisms responsible for motor coordination.

Future studies should aim at quantifying coordination and proprioception deficits following immobilization. These changes have received some attention, but not nearly as much

as strength deficits and muscle atrophy (Opie, Evans, Ridding, & Semmler, 2016). Our work provides a rationale for more research to be conducted to assess changes in coordination as well as changes in connectivity, both at rest and during different task, following immobilization. No outcome in the present study aimed at measuring the change in motor coordination and performance during the immobilization period. This could be the main reason why no significant correlations were observed. Indeed, the rs-FC changes involved regions mainly responsible for motor coordination, while the other outcomes focused on motor drive, strength, and voluntary activation. Thus, future studies should look at how a change in performance on a certain motor coordination task correlates to changes in rs-FC, which could provide an even better insight on the role of the changes observed in the present study.

The secondary analysis also provides some future directions for studies aiming to further assess rs-FC in immobilization. Of the 15 additional clusters obtained with the less conservative threshold, five involved the cerebellum (bilaterally), and six involved the thalamus (bilaterally). These results follow the findings presented in the primary analysis, which also involved these two regions. Additional directions provided by the secondary analysis are the different clusters involving primary sensorimotor regions. Although the hypothesis of segregation suggested by Newbold and colleagues (2020) was not supported by our results, modest changes in connectivity still occurred in the immobilized M1, the immobilized S1, and the non-immobilized S1, further supporting the potential for neuroplastic changes in the sensorimotor network during limb immobilization.

It is important to note that this study had some limitations. First, because the primary outcomes of this study were muscle strength and corticospinal excitability, the sample size was limited to 12 participants, which may not provide enough statistical power to identify all the changes in rs-FC that occurred over the immobilization period, and which may be why so many trends were identified at a threshold of $Z > 2.6$. Furthermore, clinical outcomes such as muscle

strength and voluntary activation were correlated to changes in rs-FC, but no correlation was significant after correction, which could be partly due to this small sample. To reduce the burden on the participants, they were also allowed to use their immobilized hand. Because the absence of sensorimotor information was limited to the elbow joint, it may explain why the M1 and S1 underwent more modest changes in connectivity. A longer or more restrictive immobilization period, or simply a larger sample size, could potentially allow to identify more significant changes in brain rs-FC, as suggested by the trends identified at a threshold of $Z > 2.6$.

3.6 Conclusion

Overall, the results of the present study show that brain regions involved in coordination, motor learning, and error detection were the most affected by a two-week upper-limb immobilization. This should be taken into account in future studies, which should look at how this correlates to losses in coordination and motor skill performance.

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3.8 Supplementary Material

Supplementary Material Table 1. Correlations Between Changes in rs-FC and Clinical Outcomes

Clusters		Clinical Outcomes							
Seeds	Regions	Immo. Arm Flexion		Non-Immo. Arm Flexion		Immo. Arm Voluntary Activation		Non-Immo. Arm Voluntary Activation	
		r	p	r	p	r	p	r	p
Left Cerebellum Lobule VIIa	Left Middle Temporal Gyrus	.120	.711	-.262	.410	-.010	.976	-.490	.126
	Bilateral SMA	.093	.773	-.174	.589	.452	.163	-.336	.312
Left vPMC	Right Cerebellum Lobule VIIb	.770	.003	.496	.101	.249	.461	-.343	.301
Right VL	Right Posterior Insula	-.258	.419	-.149	.643	-.086	.802	-.094	.784

Significance was set at $p < 0.003$ according to Bonferroni correction for multiple comparisons.

SMA: supplementary motor area, vPMC: ventral premotor cortex, VL: ventrolateral nucleus of the thalamus.

Supplementary Material Table 2. Mean torque and voluntary activation

Clinical Outcomes								
Arm	Flexion MVC (Nm)		Voluntary Activation (%)		Superimposed Twitch (Nm)		Resting Twitch (Nm)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Immobilized	32.0	24.7	92.8	88.5	0.346	0.533	4.996	4.237
Non-Immobilized	34.9	32.8	96.6	97.1	0.159	0.193	4.435	5.288

MVC: maximal voluntary contraction, Nm: Newton x meters.

Supplementary Material Table 3. Significant changes in rs-FC with a threshold of $Z > 2.6$

Contrasts	Clusters								
	Seeds	Regions	Brodmann Areas	Size (voxels)	MNI Coordinates			Max Z-value	p
					X	Y	Z		
Pre > Post	Left Lobule X	Left Middle Frontal Gyrus	N/A	2714	-29	45	-5	3.84	0.010
	Right Lobule VIIla	Right Inferior Parietal Lobule	N/A	2364	33	-48	53	3.71	0.015
	Left Lobule VIIla	Left Middle Temporal Gyrus	37	3591	-54	-69	6	4.52	0.001
		Left Superior Temporal Gyrus	N/A	1993	-59	-47	13	4.94	0.042
	Right VL	Left Inferior Frontal Gyrus	45	2160	-60	24	9	4.40	0.029
		Right Lingual Gyrus	N/A	2182	6	-76	-13	4.24	0.027
	Left Thalamus	Left Middle Frontal Gyrus (dlPFC)	46	2955	-45	15	26	4.59	0.004
Pre < Post	Right M1	Right Posterior Cingulate Cortex	N/A	1931	9	-42	21	4.25	0.043
	Right S1	Right Superior Temporal Gyrus	N/A	2363	59	-51	20	4.23	0.020
	Left S1	Right Anterior Cingulate Cortex	32	2826	17	46	10	4.17	0.012
	Left vPMC	Right Cerebellar Crus 2	N/A	4521	8	-76	-38	4.52	<0.001
	Right dPMC	Left Medial Frontal Gyrus	N/A	2907	-9	35	-12	4.24	0.006
	Right Lobule X	Left Sub-Gyral Region	N/A	2226	-46	-7	21	3.91	0.026
	Left Lobule VI	Left Posterior Cingulate Cortex	23	2317	-2	-56	16	3.81	0.010
	Left Lobule VIIla	Precuneus Area	N/A	3272	0	-58	21	3.9	0.001
		Medial Frontal Gyrus (SMA)	6	4665	-14	-12	59	4.26	<0.001
	Right VL	Right Insula	13	2921	40	-4	15	5.05	0.005
	Right VA	Right Insula	13	2119	37	-2	14	4.63	0.041
	Left Thalamus	Right Cingulate Gyrus	N/A	2589	11	-5	45	3.76	0.009

MNI: Montreal Neurological Institute, VL: ventrolateral nucleus of the thalamus, dlPFC: dorsolateral prefrontal cortex, M1: primary motor cortex, S1: primary somatosensory cortex, vPMC: ventral premotor cortex, dPMC: dorsal premotor cortex, SMA: supplementary motor area, VA: ventroanterior nucleus of the thalamus

Chapter 4: Scholarly Discussion

The results of the present study suggest that the connectivity between regions of the brain involved in error detection as well as motor learning and coordination are the most affected by a fourteen-day upper-limb immobilization. While this was not expected based on the information present in the current literature regarding brain function and immobilization, it provides some insight on the mechanism underlying the previously described losses in coordination associated with limb immobilization (De Marco et al., 2021; Moisello et al., 2008).

The current results also raise a flag regarding rehabilitation following an immobilization period. Indeed, most rehabilitation programs are based on regaining strength after immobilization (Moseley et al., 2015). While focusing on restrengthening the immobilized limb makes sense based on the effects of immobilization on strength levels, the results of the present study suggest that relearning how to move properly might be just as important because of the decreased ability to detect errors. In running for example, specific kinematic patterns can lead to injury, and relearning has been shown to have long-lasting change on running biomechanics and reduce the risk of injury (Doyle, Doyle, Bonacci, & Fuller, 2022). In sports such as tennis, using suboptimal biomechanics can also lead to shoulder and back injuries (Elliott, 1988, 2006). Thus, following an immobilization period, if motor coordination has been affected and errors cannot be detected as effectively like suggested by the results presented in this study, it is important to re-establish appropriate biomechanical habits and focus on relearning the movements in order to avoid further injuries. Hopefully, by focusing on biomechanics and technique in conjunction with regaining the strength and mass lost, error detection capacity and pre-immobilization rs-FC could be restored. Techniques such as video feedback have been shown to be an effective training strategy (Popovic et al., 2018) and can thus be used, along with coaching, in order to further support the error detection

functions of the brain. While the first step to take may be strength training after an extended immobilization period, coordination and biomechanics training should occur as well. A rehabilitation program combining both strength and motor learning could thus help prevent injuries and regain the loss in error detection and coordination.

It would also be insightful to manipulate this study slightly in order to gain more insight on exactly how the brain reacts to different contexts of immobilization. First, it would be interesting to look at the changes in rs-FC caused by the same type of immobilization, but in different populations. While the participants in this study were healthy, they were not highly trained. For instance, a group of individuals with weight lifting experience would be interesting to look at. It is well-known that weight training has an important effect on neural drive (Del Vecchio et al., 2019) and that reducing or stopping a training program produces detraining effects, subsequently causing a decrease in that neural drive (Gondin, Guette, Ballay, & Martin, 2006). Thus, the neural drive of a group that has a stronger baseline connection between the brain and the muscles could potentially be more affected by limb immobilization, although the neural changes induced by weight training have been shown to be long lasting (Andersen, Andersen, Magnusson, & Aagaard, 2005). In this type of population, regions responsible for the direct brain output to the muscles such as M1 could have larger changes in connectivity with the rest of the brain than in the case of the participants in the present study, in which no change in M1 connectivity was observed. On the other hand, a group trained in a sport that requires more upper-limb coordination such as tennis could see an even larger difference in connectivity in the regions highlighted in the results of the present study. Thus, targeting different specific populations could provide insight on the importance of pre-immobilization conditions on the impact of the disuse on rs-FC. Such highly trained individuals, however, would be harder to recruit because of the demands of the sports and the burden that such an immobilization period can impose on an athlete.

Second, the results of a similar study looking at lower-limb immobilization would also be interesting to compare with the results of the present study, and could provide insight similar to a study comparing different populations. Because of the different daily use humans make of upper limbs in comparison to lower limbs, the effects of immobilization on rs-FC could potentially be quite different. Indeed, while arms are used for a wide variety of tasks requiring strength, coordination, and fine motor skills, legs are involved mainly in locomotion and need to support the entire body weight. The immobilization of different limbs would thus potentially also cause different changes in the brain. In sum, the results of this study provide a foundation to better understand the brain mechanisms impacted by immobilization. More research is needed to assess the impact of immobilization on different people and different limbs and to reproduce the findings currently available in the literature.

Overall, although the present study does provide information regarding the mechanisms of how loss in motor skills occurs over the course of an immobilization period, it fails to explain exactly how loss in strength usually exceeds muscle atrophy after limb immobilization. There seemed to be some trends involving the disused M1 that could potentially offer some explanation on how muscle drive might decrease following limb restriction, but no significant change at a threshold of $Z > 3.1$, the gold standard in rs-FC analysis, really involved brain regions responsible for sending the output directly to the muscles. As previously mentioned, this lack of results regarding regions involved in motor drive could be caused either by the small sample size or because participants were allowed to use the hand of the immobilized arm, potentially attenuating the effect of the immobilization on the brain. It would also be interesting to further assess the connectivity of M1 during contraction, similarly to what Garbarini and colleagues (2019) achieved.

Chapter 5: Conclusion and Summary

To our knowledge, this was only the second study quantifying rs-FC in limb immobilization. While the effects of a two-week upper-limb immobilization were expected to be most present on the M1 and other brain regions involved in motor drive, the changes mainly affected regions of the brain responsible for error detection, movement adaptation, and coordination. Indeed, the cerebellum underwent three significant changes in connectivity, which seemed to show a decreased capacity to detect movement errors in the immobilized arm and an increase in error detection in the non-immobilized arm.

Future studies should look at different populations and use different immobilization paradigms (duration, limb involved) in order to assess whether the changes observed in the present study can be generalized to the entire population. It is likely that different populations and immobilization of different limbs would produce unique changes in rs-FC, but more research is needed to make any sort of conclusion.

Overall, the present study provides a very solid basis for understanding and explaining the mechanisms underlying the loss in motor coordination that occurs during limb immobilization, but offers very little information to better understand the loss in strength. Hopefully future studies will be able to observe the same changes in terms of error detection, correlate these effects to behavioral changes in fine and gross motor skills, and better explain changes in muscle strength following limb immobilization.

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