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Cortical Mechanisms of Human Pelvic Floor Muscle Synergies

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Cortical Mechanisms of Human Pelvic Floor Muscle Synergies

by

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Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.

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DEDICATION

I dedicate this PhD to my parents who are the reason why I am here today. My mother especially, is the one who has instilled such a drive in me; I am grateful for learning and appreciating what hard work is all about; a valuable lesson I hope to instill in my future children.

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ABBREVIATIONS

ACC	Anterior Cingulate Cortex
ANS	Autonomic Nervous System
APA	Anticipatory Postural Adjustments
BOLD	Blood Oxygenation Level Dependent
BA	Brodman's Area
CPPS	Chronic Prostate/Pelvic Pain Syndrome
EEG	Electroencephalography
EMG	Electromyography
FDI	First Dorsal Interosseous
FHL	Flexor Hallucis Longus
FI	Fecal Incontinence
fMRI	Functional Magnetic Resonance Imaging
GMM	Gluteus Maximus Muscles
IC	Interstitial Cystitis
LA	Levator Ani
LAI	Left Anterior Insula
LBP	Low Back Pain
MVC	Maximum Voluntary Contraction
MEP	Motor Evoked Potential
MNI	Montreal Neurological Institute
PBS	Painful Bladder Syndrome
PET	Poistron Emission Topography

PFM	Pelvic Floor Muscles
SMA	Supplementary Motor Area
UI	Urinary Incontinence
UCPPS	Urologic Chronic Pelvic Pain Syndrome

ABSTRACT OF THE DISSERTATION

Cortical Mechanisms of Human Pelvic Floor Muscle Synergies

by

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Dr. Lee Berk, Chairperson

The human pelvic floor is an anatomically, functionally, and morphologically complex region that is associated with many disorders such as chronic prostatitis/pelvic pain syndrome (CPPS), chronic low back pain, and urinary incontinence. The purpose of this dissertation was to explore the cortical mechanisms that underlie human pelvic floor muscle synergies. Our first original experiment involved the study of 20 healthy male controls who were instructed to perform a variety of muscle tasks presumed to be associated with pelvic floor muscle synergies. Surface electromyography (EMG) method was used to detect timing onsets, as well as activation patterns of the pelvic floor, gluteus maximus, and first dorsal interosseous muscles. Functional magnetic resonance imaging (fMRI) was used to measure blood oxygenation density levels (BOLD) in the brain while subjects performed various prime mover tasks. Our second original experiment involved another set of 10 healthy male subjects who were trained to perform a complex synergy breaking/decoupling task that was confirmed with EMG. They repeated the coupling motor task (gluteal activation) as well as the more complex motor decoupling task while being scanned with fMRI, so that BOLD signals could be compared. The first experiment revealed evidence of cortically facilitated synergy of the pelvic floor muscles and the

second experiment revealed that complex motor tasks such as the breaking of a cortically facilitated muscle synergy involves BOLD signals in the brain known to be involved with interoception.

CHAPTER ONE

BACKGROUND

Overview of the Human Pelvic Floor

Pelvic Floor Function

The architectural function of the pelvic floor is to support pelvic organs such as the abdominal viscera and rectum, while the functional role of the pelvic floor muscles (PFM) is to control continence and elimination (Messelink et al., 2005). The complexity in function of the PFM lies in the fact that its dysfunction has been shown to be associated with many disorders such as low back pain (LBP), CPPS, fecal and urinary incontinence (FI/UI), vulvodynia/chronic pelvic pain, and overactive pelvic floor syndrome. To logically understand how the PFM may have a role in these disorders, we first discuss the basic functional anatomy of the pelvic floor.

Pelvic Floor Functional Anatomy and Innervation

The pelvic floor is primarily made up of two layers: the striated Levator Ani Muscle complex and external anal sphincter muscle. The pelvic floor has been described as having a third layer making up the superficial layer also known as the urogenital diaphragm. The layer consists of not only the external anal sphincter, but also the bulbospongiosus, ischiocavernosus, and superficial transverse perinei (Vodušek, 2004). The levator ani consists of 4 parts: iliococcygeus, pubococcygeus, puboretalis, and coccygeus. Relaxation of these muscles allows evacuation of the bladder and rectum,

while contraction facilitates continence. The external anal sphincter consists of 3 circular loops of muscles: the musculus subcutaneous without attachment, the superficial elliptical part attached to the coccyx, and the deep part that blends into the puborectalis. It is in a state of constant tonic contraction having no identifiable antagonist, therefore assisting in the maintenance of fecal continence. The motor neurons innervating the PFM originate from the Onuf nucleus in the S2-S4 anterior horn of the spinal cord (Vodušek, 2004). The somatic fibers from the ventral rami (also called the sacral plexus) form the pudendal nerve, which ultimately contributes to motor innervation of all the muscles of the pelvic floor, as well as the external urethral sphincter. Although not directly part of the pelvic floor, the perineal nerve is the inferior and larger terminal branch of the pudendal nerve that further divides into posterior scrotal/labial and muscular branches (Vodušek, 2004). While the PFM has direct peripheral nerve innervation by portions of the sacral plexus, the autonomic nervous system contributes to the continence function of the pelvic floor at the visceral level as well. The visceral afferents accompany both parasympathetic and sympathetic efferent fibers, while the somatic accompany the pudendal nerves and direct somatic branches of the sacral plexus. This autonomic innervation results in the sympathetic nerves having the ability to facilitate an inhibitory effect on colon peristalsis and secretions, while the parasympathetic stimulation increases peristalsis and secretions. While there is a considerable amount of detailed information known in humans on the peripheral innervation of the pelvic floor, there is a need to better understand PFM function at the cortical level (Vodušek, 2004).

Pelvic Floor Cortical Representation

Since the increased use of functional magnetic resonance imaging (fMRI) in studying cortical mapping of muscle function over the past decade, there has been an increasing trend in understanding the cortical representation of various muscles. Of particular interest, is better understanding muscle synergies of the human pelvic floor at the cortical level. To begin, it is important to understand what is currently known in terms of cortical representation of the human pelvic floor. Three neuroimaging studies which investigated brain activity during voluntary contractions of the pelvic floor in healthy controls came to divergent conclusions (Blok et al., 1997; Vodušek, 2004; Zhang et al., 2005; Seseke et al., 2006b). Blok et al. found activity in the superolateral and superomedial precentral gyrus (primary motor cortex), during repeated pelvic floor straining in healthy women. Zhang et al. did not find M1 activity but reported strong activity in the supplementary motor area (SMA), as well as parietal cortex, limbic system, cerebellum, and putamen; especially in the full-bladder condition, while contracting the PFM. Seseke et al. found that relaxation and contraction of the PFM resulted in activation patterns that included both M1/S1 and SMA, as well as the frontal cortex, cerebellum, and basal ganglia. Despite the divergent findings from the given studies, there is a strong presence of pelvic floor muscles being consistently represented in the SMA. Because our interest lies within muscle synergies and the mechanisms by which muscle synergies affect clinical conditions such as LBP, CPPS, and FI/UI (fecal incontinence/urinary incontinence), we have chosen to exploit the area of functional imaging to further understand human muscle synergies of the pelvic floor.

CHAPTER TWO

INTRODUCTION

Pelvic Floor Associated Disorders

Preserving dignity at the humane level by maintaining continence is accomplished through proper functioning of the pelvic floor muscles. However, when the PFM becomes impaired, other disabling consequences may ensue. The most obvious of consequences is urinary incontinence (UI) or fecal incontinence (FI). According to the most recent Cochrane review in 2010, there is strong support for the widespread recommendation that pelvic floor muscle training be included as first-line conservative management programs for women with stress, urge or mixed, urinary incontinence (Hay-Smith and Dumoulin, 2006). This review summarizes the important role the PFM plays in women with UI.

Along with UI or FI, there are a variety of disorders and syndromes in which an impaired PFM may be relevant. It has been shown that PFM dysfunction is associated with the development of LBP (Sjödahl et al., 2009; Arab et al., 2010). Bi et al. published a randomized clinical trial to assess the effect of pelvic floor muscle exercise in patients with chronic low back pain and found that pelvic floor exercises in combination with routine treatment provides significant benefits in terms of pain relief and disability, when compared to routine treatment along (Bi et al., 2013). PFM insufficiency is believed to occur as a result of pain, poor movement patterns, trauma, surgery, or childbirth (Sjödahl et al., 2009). These muscle imbalances do not recover and can lead to sustained

inappropriate muscle synergies that may largely contribute to the chronic pain vortex. Whether pain is a precursor to PFM impairments or vice-versa leaves us with a "chicken or the egg" question. Demystifying the complexity of pain as it pertains to the pelvic floor remains a challenge.

Another PFM associated disorder is chronic pelvic/prostate pain syndrome or any nebulously labeled pain syndrome such as vulvodynia, overactive pelvic floor syndrome, spastic levator ani syndrome, pubalgia, or simply put – urologic chronic pelvic pain syndrome (UCPPS). The mechanisms underlying chronic pelvic pain remains largely unknown and has resulted in a diagnosis of exclusion, such as: ruling out factors related to psychosocial, genitourinary system, organ specific, infection, neurologic/systemic, and/or skeletal muscles (Doggweiler and Stewart, 2011). Descriptive studies that allude to any such proposed mechanisms are scarce. However, there is evidence for the use of muscle inhibiting agents such as botulinum toxin injection therapy for patients thought to have pain from spastic PFM or PFM with trigger points (Abbott et al., 2006; Abbott, 2009). Unfortunately, results comparing favorable outcomes were not much different than placebo groups in one pilot study as well as one of the randomized clinical trials (Bø et al., 2009). The treatment of PFM with tone inhibiting agents might imply that there is an up-regulated tonus of the PFM group relative to its synergistic muscles. For this reason, we turn to the conceptual framework of understanding muscle synergies of the pelvic floor and exploring the synergistic relationships between the PFM group and other larger muscle groups.

Muscle Synergies of the Human Pelvic Floor

There is a large body of literature demonstrating that muscles of the human pelvic floor - that is, muscles of the perineum and rectum that contribute to the control of urination, defecation, and sexual activity are activated synergistically with other muscles during functional tasks. For example, pelvic floor muscles activate during voluntary contraction of abdominal muscles (Hodges, 1999; Sapsford and Hodges, 2001; Critchley, 2002; Bø et al., 2003; Sapsford, 2004; Hodges et al., 2007; Smith et al., 2007; Madill and McLean, 2008; Sjödahl et al., 2009), gluteal muscles (Bø and Stien, 1994; Peschers et al., 2001), hip adductors (Bø and Stien, 1994), and even voluntary shoulder flexion or extension (Hodges et al., 2007; Sjödahl et al., 2009). Pelvic floor muscle synergies have been suggested to be an important mechanism to promote continence when functional tasks generate increased intra-abdominal pressure (Junginger et al., 2010).

Despite multiple larger muscle groups that have been shown to co-contract with the PFM group, the deep abdominal muscles (transverse abdominis) and PFM pair have been most recently and prevalently studied because of their synergistic activity during normal trunk activities (Bø et al., 2009). Critchley et al. have shown a direct co-contraction of the transversus abdominis muscle when the pelvic floor is the prime mover (Critchley, 2002). In 2006, Madill et al. also found that abdominal muscles co-contracted and intravaginal pressure also increased when healthy continent women were instructed to perform pelvic floor contractions (Madill and McLean, 2006). Sapsford et al. found congruent results in a similar experiment they performed on healthy controls quantifying PFM activity and abdominal activity during exercises for the PFM in various positions using fine-wire electromyography (EMG). Furthermore, they also included a small pilot

in which they instructed the two subjects to perform various abdominal maneuvers. It was from this pilot that they found the subjects had an increase EMG activity of the pubococcygeus muscle as a co-contraction to the primary abdominal muscles. Ultimately they concluded that activation of the abdominal muscles was a normal response to PFM exercises. The mechanism by which there is muscle synergy between the PFM and abdominal muscles is explained by the pelvic floor muscles' contribution to intra-abdominal pressure and trunk stability. Hodges et al. have also demonstrated a feed-forward mechanism by which the PFM may be activated because of an anticipatory postural adjustment (APA) in response to trunk perturbation, such as rapid arm movement (Hodges et al., 2002). Of all larger muscle groups, the transverse abdominis is the only one that has been studied for the treatment of urinary incontinence in conjunction with or without the PFM (Bø et al., 2009).

The other large muscle group where literature has shown sparse hints of synergistic contraction with the PFM is the gluteus maximus muscles (GMM). Solianik et al. have shown functional and morphological connections between the gluteus maximus and PFM during voluntary contraction of the PFM (Soljanik et al., 2012). They demonstrated that Levator Ani (LA) and GMM contractions were electromyographically observed in 97.2% of their subjects when GMM was the prime mover. Structural mapping of the LA, GMM, and connecting fascia of the fossa ischioanalis showed synchronous movement of all structures during pelvic floor contraction. Although they did not claim this to be a synergistic contraction via any neural connectivity, they concluded that the LA and GMM are functionally and morphologically connected; calling it the 'LFG-Complex.' They further recommended considering the integration of

this complex as part of the pelvic floor. It is also noted that they did instruct their subjects to perform maximum contractions of the PFM and this may have resulted in a compensatory over-recruitment mechanism of synergistic muscles. It is possible that they may have only recorded isolated PFM contraction if their subjects were instructed to perform light voluntary contractions. To further support the synergistic coupling of the PFM and GMM, Peschers et al. evaluated PFM strength using four different techniques (Peschers et al., 2001). They found that the combined contraction of the GMM and PFM resulted in significantly increased strength readings compared to contraction of the PFM alone. This supports the notion of a functional synergy between these two muscle groups. Bo et al. found clear co-contractions of the PFM while the following prime mover muscles were contracting: gluteus maximus, abdominals, and hip adductors (Bø and Stien, 1994). Schrum et al. was able to show activity of the PFM contraction that was independent of the GMM or flexor halluces longus, which was set to be the control muscle comparison (Schrum et al., 2011b). In summary, there appears to be a synergistic coupling of the PFM group when the GMM is the prime mover but there is no contrary synergistic coupling when the PFM is the prime mover. To further understand how or why such a relationship might exist between the PFM and GMM, we designed and executed two original experiments on healthy male subjects that involved the use of EMG and fMRI.

Chapter Three introduces our first original experiment that seeks to understand the muscle synergies of the human pelvic floor by first employing peripheral EMG while healthy male subjects performed a variety of muscle tasks that could be replicated in a fMRI scanner. Subjects were asked to perform repeated isometric primary mover tasks

that involved collecting EMG data on the PFM, GMM, and first dorsal interosseous (FDI). Onsets and magnitudes of the various muscles were processed and analyzed to determine what muscle synergies of the pelvic floor, if any, existed. Subjects repeated all muscle tasks in the fMRI scanner so that BOLD signals could be measured and correlated with EMG findings. Comparing EMG data to fMRI data may help determine if muscle synergies of the pelvic floor are cortically facilitated. Following this presumption that muscle synergies of the pelvic floor do exist, we planned and executed our second original experiment that is introduced in Chapter Four.

Chapter Four introduces the pelvic floor decoupling experiment in which subjects go through a training program to teach them how to break the synergy between the PFM and the GMM. Once they have been confirmed to have the capability of breaking the synergy between the PFM and GMM with objective EMG confirmation feedback, they proceeded to perform the same tasks in an fMRI scanner. FMRI was used to compare a coupling task of the GMM/PFM and the complex decoupling task in which subjects were asked to maintain GMM contraction while shutting off or down-regulating any PFM contraction. Understanding the neural correlates of a naturally occurring coupling task versus a more complex learned motor behavior task may help better interpret cortical mechanisms related to the learning of more complex motor coordination behavior activities.

CHAPTER THREE

CORTICALLY FACILITATED MUSCLE SYNERGIES OF THE HUMAN PELVIC FLOOR

Abstract

Human pelvic floor muscles have been shown to operate synergistically with a wide variety of muscles, which has been suggested to be an important contributor to continence and pelvic stability during functional tasks. However, the neural mechanism of pelvic floor muscle synergies remains unknown. Here we tested the hypothesis that activation in motor cortical regions associated with pelvic floor activation is part of the neural substrate for such synergies. We first use electromyographic recordings in 10 healthy males to extend previous findings and demonstrate that pelvic floor muscles activate synergistically during voluntary activation of gluteal muscles, but not during voluntary activation of finger muscles. We then show, using functional magnetic resonance imaging (fMRI) in 10 healthy males that a region of the medial wall of the precentral gyrus consistently activates during both voluntary pelvic floor muscle activation and voluntary gluteal activation, but not during voluntary finger activation. We finally confirm, using transcranial magnetic stimulation in 10 healthy males, that the fMRI-identified medial wall region is likely to directly facilitate pelvic floor muscle activation. Thus, muscle synergies of the human pelvic floor appear to be facilitated by activation of motor cortical areas.

Introduction

There is a large body of literature demonstrating that muscles of the human pelvic floor - that is, muscles of the perineum and rectum that contribute to the control of urination, defecation, and sexual activity - are activated synergistically with other muscles during functional tasks. For example, pelvic floor muscles (PFM) activate during voluntary activation of abdominal muscles (Sapsford et al., 2001; Madill and McLean, 2008), gluteal muscles (Bø and Stien, 1994; Peschers et al., 2001), hip adductors (Bø and Stien, 1994), and even voluntary shoulder flexion or extension (Hodges et al., 2007; Sjödahl et al., 2009). PFM synergies have been suggested to be an important mechanism to promote continence by resisting increased intra-abdominal pressure generated by functional tasks (Junginger et al., 2010) .

Despite the potential relevance of PFM synergies to prevalent clinical conditions, including incontinence (Bø and Stien, 1994; A. Ashton-Miller, 2001; Sapsford et al., 2001; Parekh et al., 2003) and chronic pelvic pain (Doggweiler-Wiygul and Wiygul, 2002; Doggweiler-Wiygul, 2004), the neural mechanism of these synergies is poorly understood. While many muscle synergies are likely shaped by subcortical connections (Mussa-Ivaldi et al., 1994; Saltiel et al., 2001; Cheung et al., 2009), there is evidence of cortical involvement in structuring muscle synergies (Mussa-Ivaldi et al., 1994; Saltiel et al., 2001; Drew et al., 2008; Cheung et al., 2009; Waters-Metenier et al., 2014). Synergistic pelvic floor activity has been shown to occur in advance of activity in the primary muscles used to complete a task (Sapsford and Hodges, 2001; Hodges et al., 2007), suggesting that PFM activity may be part of a feedforward synergy. Since extensive research has demonstrated the cortical underpinnings of feedforward synergies

(Aruin, 2002; Jacobs et al., 2009) we hypothesized that PFM synergies may be facilitated by activity in specific motor cortical areas that enhance PFM activation.

A long line of evidence, dating back to at least Leyton and Sherrington (1917), demonstrates that pelvic floor musculature is represented in the medial wall of the precentral gyrus, primarily in Brodmann area (BA) 6 (Leyton and Sherrington, 1917; Turnbull et al., 1999; Schrum et al., 2011b). We hypothesized that, if pelvic muscle synergies are cortically facilitated, that there would be a medial wall region that was active during voluntary pelvic floor activation and voluntary activation of synergists, and that moreover, stimulation of this region would facilitate pelvic floor activation. Using a combination of electromyographic (EMG) recording, functional magnetic resonance imaging (fMRI), and transcranial magnetic stimulation (TMS), we present data below in support of this hypothesis.

Methods

Participant Population

We recruited 32 healthy men with a mean age (± SD) of 32.63 ± 5.89 (range 24 to 43). Since possible sex differences in the control of PFM have not been fully characterized, we limited our study to a single sex as in previous studies (Seseke et al., 2006b; Schrum et al., 2011b). Participants were practicing physical therapists or physical therapy students with general knowledge of pelvic floor anatomy and function. The studies we describe here were carried out at the University of Southern California and approved by the University of Southern California Institutional Review Board. All participants provided informed consent.

EMG Acquisition and Analysis

In 10 participants, we measured muscle activation using EMG to define the characteristics of PFM synergies before performing the same tasks using fMRI to define the neural substrate. We used EMG to verify the previously-reported muscle synergy between the PFM and gluteus maximus muscle (GMM) and to establish finger muscle activation as an appropriate control muscle group that does not have synergistic coupling with the PFM muscles. With the participant resting in a supine position inside a mock magnetic resonance imaging (MRI) scanner, we recorded surface EMG data from the right GMM, the PFM, and the right first dorsal interosseous muscle (FDI). We recorded EMG signals from the GMM and FDI with miniature electrode/preamplifiers (DELSYS, Boston, MA) with 2 silver recording surfaces, 5mm long and 10 mm apart. We recorded an aggregate EMG signal from the PFM using a medical-grade rectal EMG sensor (The Prometheus Group, Dover, NH). The EMG preamplifier filters had a bandwidth of 20-450 Hz, with gains of 1000 for GMM and FDI, 10000 for PFM, and a sampling rate of 2000 Hz.

Prior to the experimental session, we asked participants to empty their bladder. Participants performed separate trials, each of which involved voluntary activation of a different primary muscle group. In PFM trials, we instructed participants to contract their pelvic floor as if to stop the flow of urine. In GMM trials, we instructed participants to isometrically contract their GMM. In FDI trials, we instructed participants to contract their FDI muscle to generate index finger abduction. For all trial types, we first acquired EMG data corresponding to maximal voluntary contraction (MVC). During subsequent trials, participants activated the appropriate muscle group according to an audio tone that

ramped up and down in frequency to guide the participant through a smooth activation over a period of 2 seconds. Each trial consisted of 2 blocks of 10 activations. Previous studies of brain activity during PFM activation have not used EMG in the scanner (Seseke et al., 2006b; Kuhtz-Buschbeck et al., 2007; Schrum et al., 2011b). Since we planned to repeat voluntary activation trials in the fMRI scanner without EMG, we instructed participants in the mock scanner EMG study to produce moderate muscle activation (approximately 20% effort) to avoid fatigue, and quantified the activation (expressed as % MVC).

We analyzed EMG data to first estimate the activation onsets of the primary muscle group of each trial, and then to determine if significant time-locked activity occurred in EMG signals from the other recorded muscles. To perform this analysis, EMG signals from all recorded muscles were first high-pass filtered at 100 Hz (4th order zero-lag Butterworth filter), rectified, low-pass filtered at 30 Hz (Hodges et al., 2007) and then normalized to identically processed EMG data from the maximum activation trial. EMG data were then smoothed with a 500 ms moving average. Activation onsets were defined to occur when the smoothed EMG exceeded 2 standard deviations of the EMG baseline noise with the muscle at rest. Within each participant, we then defined an *EMG transient* for each muscle and each trial by averaging the rectified and filtered EMG data across repeated muscle activations within a time window spanning 1 second before to 3 seconds after the activation onset of the primary muscle for the trial. To define significant EMG magnitude changes, we performed group statistics on the maximum of the EMG transient for muscles of interest within each participant. To define significant temporal shifts between EMG signals, we quantified temporal shifts in each participant by

determining the maximum cross-correlation between a pair of EMG transients (normalized to their maximum value).

fMRI Acquisition and Analysis

In 14 participants, we measured brain activation associated with the voluntary muscle activation tasks (described above) using fMRI. We used a 3 Tesla (GE Signa Excite) with an 8-channel head coil. We positioned participants supine viewing a fixation crosshair, and placed foam pads to limit head motion. As in previous fMRI studies of PFM activation (Schrum et al., 2011b), we collected T2-weighted echo planar image volumes with blood oxygen level dependent (BOLD) contrast (echo time 34.5 milliseconds, flip angle 90 degrees, field of view 220 mm, pixel size 3.43 mm) continually every 2.5 seconds during 3 imaging runs. Each volume consisted of 37 axial slices (3 mm slice thickness, 0.5 mm interslice gaps) that covered the brain from vertex to cerebellum. We additionally acquired a T1-weighted high-resolution anatomical image from each participant. We cued participants to voluntarily activate each muscle group (to approximately 20% effort) in 3 separate runs - PFM activation run, GMM activation run, and FDI activation run - as described above with the exception that participants performed additional activation blocks (6 blocks of 10 activations) in the scanner. All 14 participants performed PFM activation runs, 12 participants performed GMM activation runs, and 10 participants performed FDI activation runs.

We preprocessed each participant's fMRI data using the FMRIB Expert Analysis Tool (FEAT, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/), which included skull extraction using the brain extraction tool (BET) in FSL, slice timing correction, motion correction, spatial

smoothing using a Gaussian kernel with full-width half-maximum of 5 mm and nonlinear high-pass temporal filtering (100 s). We used a general linear model (GLM) to examine the changes in BOLD signal associated with muscle activation for the three tasks. We performed participant-level whole-brain GLM analyses of individual runs in each participant to determine the change in BOLD signal during the activation blocks compared to the rest blocks. We then performed a group-level mixed-effect (FLAME 1 in FSL) analysis, with unpaired 2-sided *t* tests, to identify voxels in standard Montreal Neurological Institute (MNI) coordinates with significant differences in response based on the muscle group being voluntary contracted by the participant. We thresholded group-level images with cluster-based correction for multiple comparisons with $Z > 2.3$ and $p < 0.05$. We made inferences about specific Brodmann areas using the Jülich Histological Atlas within FSL (Eickhoff et al., 2005).

TMS Acquisition and Analysis

In 8 participants, we obtained motor evoked potentials (MEP) from the PFM, with participants resting supine, using a single-pulse magnetic stimulator (Magstim 2002, The Magstim Company Ltd, Withland, UL) with a 110 mm double cone coil. We sampled EMG signal at 16000 Hz, band pass filtered at 1 to 1000 Hz, and amplified at a gain of 9500. Our fMRI findings and previous studies have shown that pelvic floor musculature is represented in the medial wall (Leyton and Sherrington, 1917; Schrum et al., 2011b). To localize the PFM representation in the anterior-posterior direction, we stimulated along the midline. We identified a participant-specific midline and central sulcus location by registering the participant's head with their T1-weighted 3D high-resolution

anatomical image using Brainsight Frameless (Rogue Research Inc., Montreal, Canada). We then used Brainsight to guide TMS coil position to the midline and to record anterior-posterior position in MNI coordinates. We applied stimulations at 7-9 locations (based on the shape and size of participant's head), one cm apart, with the most posterior location at 2 cm posterior of the central sulcus. To select an appropriate stimulus intensity for each participant, we inspected the average PFM EMG signal in response to 7 pulses at each of several sites within 2 cm of central sulcus, and selected the stimulus intensity as the minimum intensity to evoke a clearly distinguishable MEP (Tsao et al., 2008). We used average response to obtain pelvic MEPs because the PFM are active even during rest which makes it difficult to detect a small response to TMS (Mills and Nithi, 1997).

In post-analysis, we calculated the MEP magnitude as the peak-to-peak magnitude of the average MEP in the time window of 10 to 100 ms after TMS pulse onset (Pelliccioni et al., 1997; Turnbull et al., 1999; Lefaucheur, 2005). To compare among participants, we normalized MEP magnitudes with respect to the maximum MEP magnitude within each participant. For statistical analysis, we divided the stimulation locations into three location bins (posterior, middle, and anterior) along the midline. We selected the bin edges to make the middle region correspond with the precentral gyrus as defined by the Harvard-Oxford Cortical Structural Atlas in FSL. We defined the middle region by identifying the most posterior and most anterior coronal slice, which contained no voxels with non-zero probability of belonging to the precentral gyrus. Therefore, we defined posterior stimulation locations as those in the range y = -60 to -38 mm, middle y = -38 to -12 mm, and anterior y = -12 to 20 mm. We performed a 2-way ANOVA with interaction of the MEP magnitude using the factors of location bin and participant.

Results

Using recordings from the PFM, GMM, and the FDI (Figure 1A), we found that PFM activity is synergistically coupled to GMM activity, and that PFM synergistic coupling did not exist for distal upper extremity muscles such as the FDI. Example recordings from a single participant show that during repeated activation of the PFM, the GMM remained inactive (Figure 1B). However, when the participant repeatedly activated the GMM, the PFM activated in a synchronous fashion (Figure 1B). Group data of EMG transients time-locked to activation of the primary muscle demonstrated that we consistently observed this synergistic coupling of the PFM across the study population (Figure 1C). PFM activated during voluntary activation of the PFM and voluntary activation of the GMM, but not during voluntary activation of the FDI. All participants voluntarily activated their muscles, as instructed, to moderate levels. On average, participants activated PFM to 34% of maximal contraction, the GMM to 13% of maximal contraction, and the FDI to 15% of maximal contraction. Importantly, while GMM and FDI activation did not significantly differ across participants (paired t test, $p = 0.40$), PFM activation reached an average of 26% of maximal contraction during voluntary GMM activation, which was significantly greater than PFM activation during voluntary FDI activation (paired t test, $p = 0.005$) (Figure 1D). We observed that PFM activation occurred in advance of GMM activation during voluntary GMM activation (Figure 1E). Cross-correlating the average EMG transient from the PFM and from the GMM during voluntary GMM activation, we found that activation in PFM led GMM activation by an average of 128 milliseconds, which was significantly greater than 0 (maximum of 239.5 ms and minimum of 30.5 ms, t test, $p = 0.001$).

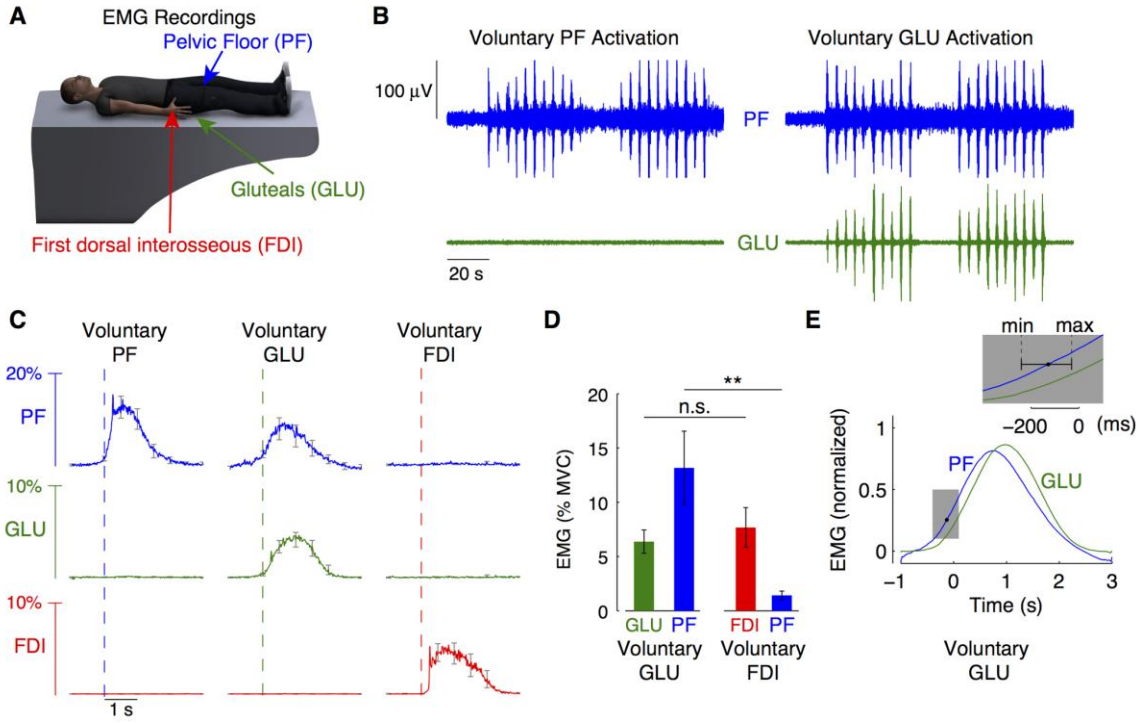

Figure 1. Electromyographic (EMG) evidence of pelvic floor muscle synergies. ***A,*** EMG signals from the pelvic floor (PFM - blue) muscles, gluteal (GMM - green) muscle, and the first dorsal interosseous (FDI - red) muscle were recorded during separate trials that focused on the voluntary activation of each of these muscle groups. ***B,*** Example EMG recordings from the PFM and GMM muscles in a single participant during repeated voluntary PFM activation and separate voluntary GMM activation. Participants performed 2 blocks of 10 activations, each activation lasting 2 seconds. We observed PFM muscle activation during voluntary GMM muscle activation, but no GMM muscle activation during voluntary PFM muscle activation. ***C,*** Group data demonstrating the consistent finding of synergistic activation of the PFM muscles during voluntary GMM muscle activation but not during voluntary FDI muscle activation. Moreover, we did not find evidence of FDI or GMM muscle activation during voluntary PFM muscle activation. Curves show the average EMG transient triggered by the onset of the primary voluntary muscle of the task, averaged across participants (error bars indicate standard error of the mean across participants). ***D,*** Statistical analysis of group data shows that PFM activity is significantly greater ($p<0.01$, **) during voluntary GMM activation compared to PFM activity during voluntary FDI activation. The activity in the primary muscles of the tasks (GMM and FDI) was not significantly different ($p=0.40$, n.s.). ***E,*** Analysis of the normalized EMG transients for the PFM and GMM muscles during voluntary GMM muscle activation revealed that activation of PFM muscles led GMM muscle activation by an average of 128 milliseconds across participants (minimum of 30.5 ms and maximum of 239.5 ms) ($p=0.001$).

Using fMRI data collected while participants performed muscle activation tasks identical to those described above (Figure 2A), we found that a region of the medial wall of the precentral gyrus activated during voluntary PFM activation and voluntary GMM activation, but not during voluntary FDI activation. We used FDI activation as a reference task in fMRI analysis because the EMG results showed that there was neither PFM nor GMM muscle activation during voluntary FDI activation. As expected, the contrast of FDI activation greater than PFM activation produced significant brain activity primarily in left sensorimotor cortex (Figure 2B), as the participant activated the right FDI. Also, as expected, the contrast of PFM activation greater than FDI activation produced significant activity in the medial wall of the precentral gyrus (Figure 2C). Surprisingly, the contrast of GMM activation greater than FDI activation, which EMG data suggest contains increased GMM activation *and* increased PFM activation, also produced significant activation in the medial wall of the precentral gyrus (Figure 2D). We found a region of the medial wall of the precentral gyrus that exhibited significant ($p>0.005$) brain activation for both voluntary PFM activation and voluntary GMM activation compared to FDI activation (Figure 2E).

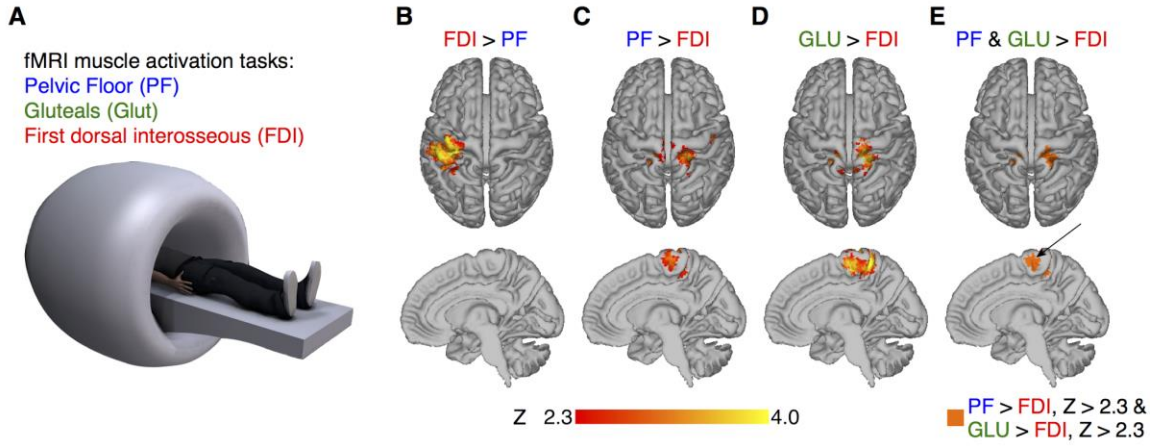

Figure 2. Functional magnetic resonance imaging (fMRI) evidence of overlapping activity during voluntary PFM and voluntary GMM activation. ***A,*** fMRI data were collected while participants performed 3 separate runs identical to the EMG tasks - separate repeated voluntary activation of PFM (run 1), GMM (run 2), and FDI (run 3). ***B,*** Contrast of voluntary FDI activation greater than voluntary PFM activation produced significant brain activation in left sensorimotor cortex. ***C,*** Contrast of voluntary PFM activation greater than voluntary FDI activation produced significant activation in the medial wall of the precentral gyrus. ***D,*** Contrast of voluntary GMM activation greater than voluntary FDI activation produced significant activation in the medial wall of the precentral gyrus ***E,*** Anterior medial wall of the precentral gyrus exhibited significant brain activation, for both PFM activation and GMM activation compared to FDI activation**.**

Using MEP data generated by application of TMS along the midline of the participant's brain (Figure 3A), we verified that medial wall of the precentral gyrus, identified using fMRI to be active during both PFM activation and GMM activation, likely facilitates PFM activation. Example data from one participant illustrates the findings (Figure 3B). Stimulation over anterior points of the midline over frontal cortex did not produce an MEP in the PFM, but stimulation over the precentral gyrus at the same stimulus intensity produced an MEP in the PFM at a latency of 23 milliseconds. In this participant, the relative magnitude of the MEP in the PFM peaked at an MNI coordinate of approximately -20 mm. The locations where we applied stimulation across all participants were confined over the midline, and were divided into posterior, middle (precentral gyrus), and anterior bins (Figure 3C). We observed that there was a significant main effect of bin location on PFM MEP magnitude, $F(2,36) = 6.62$, $p = 0.004$; no significant main effect of participant $F(7,36) = 1.24$, $p = 0.31$; and the interaction between bin location and participant was not significant $F(14,36) = 0.67$, $p = 0.79$. A post-hoc multiple-comparisons test with Bonferroni correction ($p<0.05$) indicated that MEPs corresponding to the middle bin were significantly greater than either the posterior bin ($p <0.001$) or anterior bin ($p < 0.001$) (Figure 3D). Viewing the stimulation points and the medial wall together demonstrated that stimulation points that we classified as precentral gyrus were above the fMRI-identified activation common to both voluntary PFM activation and voluntary GMM activation (Figure 3E).

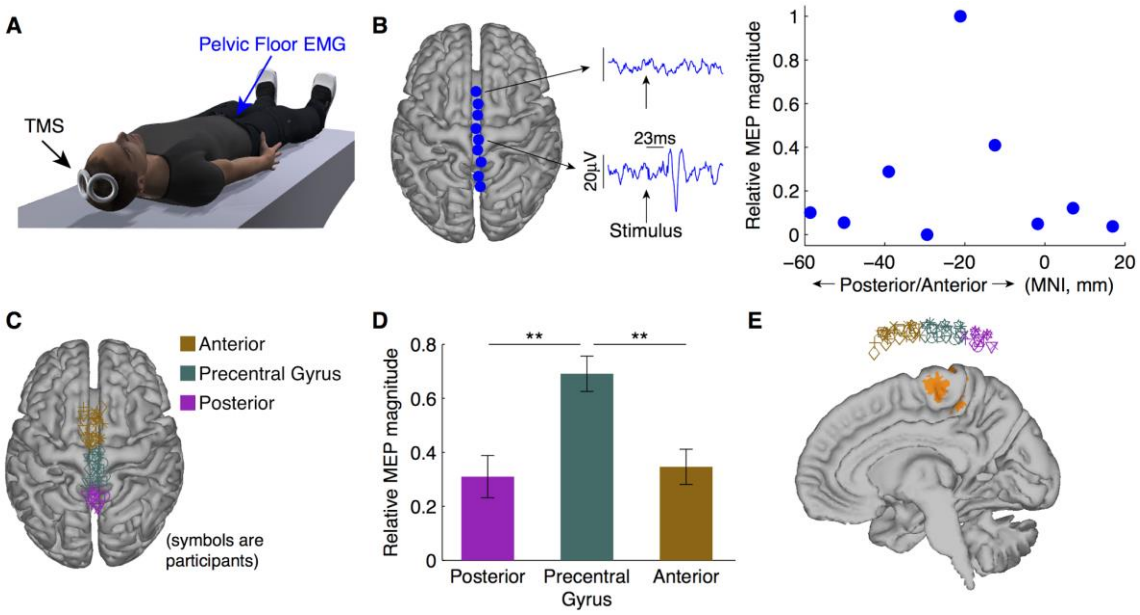

Figure 3. Transcranial magnetic stimulation (TMS) evidence that region of brain activation overlap between voluntary PFM activation and voluntary GMM activation facilitates activity in PFM muscles. ***A,*** We collected motor evoked potentials (MEP) from the PFM muscles generate by TMS along the midline of the participant's brain. ***B,*** Single participant data showing an MEP in the PFM muscles generated by stimulating over precentral gyrus at a latency of 23 ms. Stimulating at points not over the precentral gyrus did not generate significant MEPs. The MEP in the PFM peaked at an MNI coordinate of approximately -20 mm. ***C,*** Locations of applied stimulation across all participants confined over the midline and divided into posterior, middle (precentral gyrus), and anterior bins. ***D,*** MEPs corresponding to the middle bin significantly greater than either the posterior bin ($p < 0.001$) or anterior bin ($p < 0.001$) ***E,*** Stimulation points classified as precentral gyrus were above the fMRI-identified region of activation common to both voluntary PFM activation and voluntary GMM activation.

We found that the brain region with overlapping activation during both voluntary PFM and voluntary GMM activation (Figure 3E) contained contributions from both primary motor cortex (BA 4) and supplementary motor area (SMA - BA 6) (Figure 4), adding additional support to the likely motor involvement of this overlap region. We found that 36.0% of overlapping voxels were most likely BA 4, 22.3% were most likely primary somatosensory cortex (BA 1-3), 15.9% were most likely BA 6, 14.8% were superior parietal lobule (BA 5), and 10.9% were most likely corticospinal tract (CST). The foci of peak activation in the overlap region for PFM activation and GMM activation were 4.9 mm apart in primary motor cortex and 2.9 mm apart in SMA (Table 1).

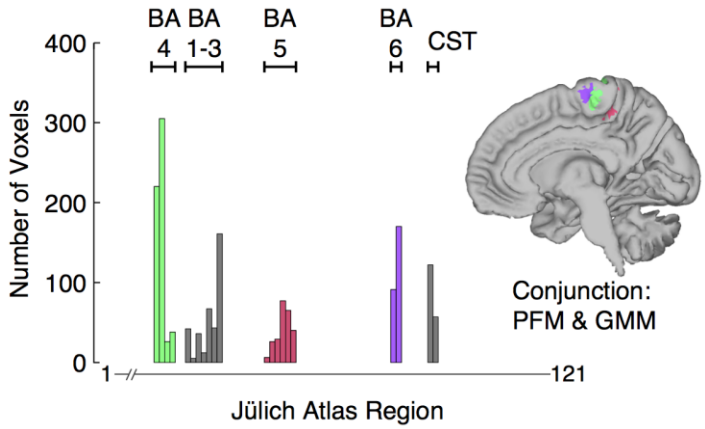

Figure 4. Evidence of motor cortical representation in the overlapping brain activation during voluntary PFM and voluntary GMM activation. We computed the number of voxels in the overlap that were most likely to belong to each of the 121 regions in the Jülich Histological Atlas within FSL. The range of atlas regions included in each Brodmann Area (BA) is labelled. CST = corticospinal tract.

Table 1: Peak foci for activation in the region of overlap obtained during voluntary PFM and voluntary GMM activation.

Region	PFM>FDI		GMM>FDI	
	Coordinates x, y, z (mm)	Z-score	Coordinates x, y, z (mm)	Z-score
Primary Motor Cortex (4)	20, -32, 64	4.70	18, -30, 68	4.86
Premotor Cortex (SMA, 6)	-4, -20, 66	3.16	-4, -22, 64	3.82
Primary Somatosensory Cortex (1-3)	-16, 34, 66	3.74	16, -36, 64	4.67
Superior Parietal Lobule (5)	-6, -40, 48	2.98	10, -40, 54	4.07

Significance: $Z>2.3$, $p<0.05$ cluster corrected for multiple comparisons. Coordinates in standard MNI space.

Discussion

Our results indicate that motor areas of the cerebral cortex may facilitate the synergistic activation of the pelvic floor that has been shown to accompany voluntary activation of hip and trunk muscles (Bø and Stien, 1994; Hodges et al., 2007). The cortical area facilitating this pelvic floor activation is the medial wall of the precentral gyrus, consistent with previous motor cortex stimulation studies in both animals and humans (Leyton and Sherrington, 1917; Turnbull et al., 1999). More specifically, this identified region in the medial wall appears to contain a clear contribution from BA 6 (supplementary motor area - SMA).

Numerous previous studies have demonstrated the importance of SMA during voluntary activation of the pelvic floor (Leyton and Sherrington, 1917; Blok et al., 1997; Zhang et al., 2005; Seseke et al., 2006b; Schrum et al., 2011b). The SMA is generally thought to be involved in higher order organization and preparation of voluntary movement (Cunnington et al., 1996). SMA has functionally and neuroanatomically distinct regions; for example, an anterior portion known as the pre-SMA, and a posterior portion known as the SMA proper (Luppino et al., 1993; Rizzolatti et al., 1996). The SMA proper contains direct corticospinal neurons (Dum and Strick, 1996), and has been shown to be involved in movement execution similar to the primary motor cortex (Macpherson et al., 1982; Picard and Strick, 1996; Boecker et al., 1998; Lee et al., 1999). Pre-SMA is thought to be more involved with motor planning associated with self-initiated tasks, and may be active even during motor imagery in the absence of movement execution (Tyszka et al., 1994; Stephan et al., 1995; Deiber et al., 1999; Cunnington et al., 2002). Our cortical mapping results of the PFM appear to coincide with SMA proper.

To our knowledge, there has been relatively little discussion regarding the functional interpretation of why the pelvic floor would have a relatively strong representation in SMA. As in previous studies of pelvic floor muscle synergies, our findings support that PFM activation occurs prior to the primary muscle of the task. Data from a variety of approaches, including electroencephalography (EEG) and fMRI, suggest that SMA activity precedes primary motor cortical activity during voluntary motor tasks (Ball et al., 1999; Soon et al., 2008; Bortoletto and Cunnington, 2010). We suggest that the SMA representation of the pelvic floor is part of the neural substrate facilitating the feedforward pelvic floor activation in advance of hip and trunk muscles as we and others have shown (Hodges et al., 2007; Sjödahl et al., 2009). If this suggestion is correct, circuits within SMA may be involved in evaluating the demands of the voluntary motor task at hand and activating the pelvic floor in preparation if necessary (as in the case of gluteal activation), or not necessary (as in the case of voluntary finger muscle activation).

Our results are consistent with suspected involvement of SMA in feedforward muscle synergies underlying postural control. Patients with SMA lesions exhibit impairments in anticipatory muscle activation (Viallet et al., 1992). Neuroimaging in healthy controls suggests there is SMA activation associated with performing anticipatory postural adjustments (APA) (Ng et al., 2013). Additionally, repetitive TMS of SMA has been shown to affect the timing of APA in both healthy controls and patients with Parkinson's disease (Jacobs et al., 2009). It has been previously suggested that PFM synergies may be part of an APA when perturbations to abdominal pressure are

predictable (Hodges et al., 2007); our work critically defines a neural substrate that may underlie these adjustments in pelvic floor muscle activation.

In this study, we have shown that a motor cortical region that facilitates PFM contraction is active during synergistic activation of the pelvic floor - we have termed this a *cortically-facilitated* synergy. Our current study conclusions are limited in scope because we have not yet established that PFM synergies are *cortically-mediated*. Cortical mediation of a PFM synergy would imply that the motor cortical area identified is *necessary* and causal for the implementation of the synergy. At present, we do not know the extent of subcortical and spinal involvement in contributing to the pelvic floor muscle synergy. For example, it is known that there are centers in the pons that facilitate PFM activity in subconscious control of urination (Fowler et al., 2008), but we do not currently know their role in contributing to the identified pelvic floor muscle synergies. Future experiments, including repetitive TMS down-regulation (Jacobs et al., 2009) of the identified medial wall region and expanded TMS mapping of PFM activation at cortical locations that activate synergistic muscles (e.g. gluteal, abdominal, shoulder), will be necessary to determine if the cortical facilitation identified in the current work can be extended to cortical mediation.

In conclusion, even though our subjects were pain-free, our results have important clinical implications for understanding motor cortical mechanisms of chronic pelvic pain. It was recently shown that women with the prevalent condition of Interstitial Cystitis / Painful Bladder Syndrome (IC/PBS) have significant changes in resting state neural activity, compared to healthy controls, in areas of the medial wall of SMA (Kilpatrick et al., 2014). The precise function of this region was not investigated in these patients, but

the authors interpreted the results in the context of possible motor control mechanisms contributing to the condition (Butrick, 2009). The results of our present study are from a male population, but major sex differences in the cortical control of the pelvic floor are not immediately suspected (Seseke et al., 2006a). The motor cortical region we have identified to facilitate PFM activation clearly overlaps with the coordinates reported for patient-specific alterations in IC/PBS. Therefore, our results may suggest that changes in motor cortical areas that make direct projections to pelvic floor motor neuron pools may play a critical role in IC/PBS pathophysiology.

CHAPTER FOUR

BRAIN ACTIVATION ASSOCIATED WITH INVOLUNTARY MUSCLE SYNERGIES OF THE HUMAN PELVIC FLOOR

Abstract

Cortical representation and functional synergies have been established for the human pelvic floor but their interaction remains unknown. We have recently identified that synergistic contraction of the pelvic floor muscles (PFM) is cortically facilitated when healthy male subjects were instructed to perform light isometric gluteus maximus muscle (GMM) contractions. The purpose of this study was to explore the neural mechanisms of training subjects to decouple this synergy. We hypothesized that there would be a blood-oxygen-level dependent (BOLD) signal difference in the motor area associated with the pelvic floor as a result of this synergy decoupling training, compared to the cortically facilitated activity of the pelvic floor when subjects were instructed to contract their GMM. In our current study, we measured regional brain activity by functional magnetic resonance imaging (fMRI) in 10 healthy males while performing two types of gluteal tasks described as being coupled or decoupled. In the coupled condition, participants were instructed to perform repeated isometric (GMM) contractions. In the decoupling condition, subjects were trained and instructed to break the gluteal/pelvic floor muscle synergy by consciously relaxing the PFM while maintaining a comfortable GMM contraction. This group was also given the following training cue: "relax the pelvic floor until sensing the urge to urinate." Our main finding was that the execution of

the coupling task as compared to the decoupling task activated the anterior cingulate cortex (ACC) and left anterior insula. We interpret that the ACC employs a modulatory effect on the primary motor cortex and the supplementary motor area (SMA), which facilitates the suppression of cortically facilitated muscle synergies. Furthermore, our findings indicate that the left anterior insula mediates somatic and visceral attention to the interoceptive state of feeling PFM relaxation or feeling the urge to urinate. These findings were not anticipated as with our original hypothesis but it does provide a unique perspective related to the role of the ACC and left anterior insula in the context of cortical mapping and functional synergies of the pelvic floor muscles. Complex motor tasks that require awareness, training, and focus on intricate somatic and visceral areas such as the pelvic floor complex may inherently require participation of the brain regions associated with interoception as well as motor control.

Introduction

Pelvic floor activity has been shown to synergistically co-contract with muscles of the hip and trunk as well as in advance of activity in the primary muscles used to complete a task (Bø and Stien, 1994; Sapsford et al., 2001; Hodges et al., 2007; Jacobs et al., 2009). This suggests that pelvic floor muscle activity may be part of a feed forward synergy in the preparation for movement, aside from its well established co-contraction type of synergy. We have recently shown this synergy to be cortically facilitated (Asavasopon et al., 2014, in submission). The cortical area facilitating this pelvic floor activation is the medial wall of the precentral gyrus. Most recent fMRI (functional magnetic resonance imaging) results and confirmatory TMS (transcranial magnetic

stimulation) findings have demonstrated that the medial wall of the precentral gyrus where the pelvic floor is distinctly found to be represented, lies within the same cortical region of the supplementary motor area (SMA) (Asavasopon et al., 2014, in submission). This is consistent with previous neuroimaging studies, which have found pelvic floor representation overlapping within the region of SMA (Blok, Willemsen, & Holstege, 1997; Seseke et al., 2006; Zhang et al., 2005). Whether or not this cortical region represents primary motor cortex (PMC) of the pelvic floor or a region that is thought to be more involved with higher organization and preparation of voluntary movement remains to be explored.

Numerous previous studies have demonstrated the importance of SMA during voluntary activation of the pelvic floor (Blok et al., 1997; Zhang et al., 2005; Seseke et al., 2006a; Schrum et al., 2011a). The SMA is generally thought to be involved in higher order organization and preparation of voluntary movement (Cunnington et al., 1996). SMA has functionally and neuroanatomically distinct regions; for example, an anterior portion known as the pre-SMA, and a posterior portion known as the SMA proper (Luppino et al., 1993; Rizzolatti et al., 1996). The SMA proper contains direct corticospinal neurons (Dum and Strick, 1996), and has been shown to be involved in movement execution similar to the primary motor cortex (Macpherson et al., 1982; Picard and Strick, 1996; Boecker et al., 1998; Lee et al., 1999). Pre-SMA is thought to be more involved with motor planning associated with self-initiated tasks, and even during motor imagery in the absence of movement execution (Tyszka et al., 1994; Stephan et al., 1995; Deiber et al., 1999; Cunnington et al., 2002). Our cortical mapping results of the pelvic floor muscles appear to coincide with SMA proper. It is interesting that the cortical

representation of premovement activity happens to be closely associated with pelvic floor muscle activation or motor execution. Although the cortical representation and functional synergies of the pelvic floor have been established, their interaction remains unknown.

To further understand functional synergies of the pelvic floor muscles (PFM), we turn to recent work demonstrating how PFM activity is cortically facilitated. In a study by Asavasopon et al., PFM EMG (electromyography) activity was shown to inherently accompany voluntary gluteus maximus muscle (GMM) activation in healthy male participants, but the reverse was not found (Asavasopon, 2014). After repeating the same tasks in the fMRI scanner, we found GMM cortical representation to overlap with PFM on the medial wall of the precentral gyrus, when GMM was the primary mover, but when PFM was the primary mover, there was no overlap with the GMM. Our TMS results further supported the notion that PFM synergy was in fact, cortically facilitated during GMM activation, as we were able to elicit PFM motor evoked potentials (MEPs) without GMM MEPs. In summary, voluntary GMM contraction is coupled with naturally occurring PFM activity, while PFM are inherently decoupled from the GMM when PFM is the primary mover.

The purpose of this experiment is to determine if cortically facilitated synergies of the pelvic floor muscles can be modified, with corresponding changes in motor cortical activity. Neural correlates during the voluntary modification of the pelvic floor muscle synergies are also hypothesized to show an increase in BOLD signal in the somatomotor areas of the brain.

Methods

Participant Population

We recruited 10 healthy men with a mean age (± SD) of 32.6 ± 5. 9). Since possible sex differences in the control of PFM have not been fully characterized, we limited our study to a single sex as in previous studies (Seseke et al., 2006a; Schrum et al., 2011a). Participants were practicing physical therapists or physical therapy students with general knowledge of pelvic floor anatomy and function. The studies we describe here were carried out at the University of Southern California and approved by the University of Southern California Institutional Review Board. All participants provided informed consent.

EMG Acquisition and Analysis

In 10 participants, we took measurements of muscle activation, using EMG, to confirm the characteristics of PFM synergies, train the participants to suppress the natural PFM activity that naturally occurs during GMM activation, and to train the participants on what to expect during the fMRI portion of the experiment. Total training time of 1 hour was given to each participant before proceeding to the actual fMRI scanner to perform the same tasks again. Participants were trained with EMG visual feedback of their PFM and GMM. We used EMG to verify the previously reported muscle synergy between the PFM and GMM, as well as to verify that the participant could appropriately perform a GMM contraction while minimizing PFM contraction as much as possible. With the participant resting in a supine position inside a mock MRI scanner, we recorded surface EMG data from the right GMM and PFM. We recorded EMG signals from the

GMM with miniature electrode/preamplifiers (DELSYS, Boston, MA) with 2 silver recording surfaces, 5mm long and 10 mm apart. We recorded an aggregate EMG signal from the PFM using medical-grade rectal EMG sensor (The Prometheus Group, Dover, NH). The EMG preamplifier filters had a bandwidth of 20-450 Hz, with gains of 1000 for GMM and FDI and 10000 for PFM, and a sampling rate of 2000 Hz.

Prior to the experimental session, we asked participants to empty their bladder. Participants performed separate trials, each of which involved voluntary activation of a different primary muscle group. In PFM trials, we instructed participants to contract their pelvic floor as if to stop the flow of urine. In GMM trials, we instructed participants to isometrically contract their GMM. In FDI trials, we instructed participants to contract their FDI muscle to generate index finger abduction. In decoupling trials, we instructed participants to contract their GMM and to "immediately relax the PFM until feeling the urge to urinate. During subsequent trials, participants activated the appropriate muscle group according to an audio tone that ramped up and down in frequency to guide the participant through a smooth activation over a period of 2 seconds. Each trial consisted of 2 blocks of 10 activations. Previous studies of brain activation during PFM contraction have not used EMG in the scanner (Seseke et al., 2006a; Kuhtz-Buschbeck et al., 2007; Schrum et al., 2011a). Since we planned to repeat voluntary activation trials in the fMRI scanner without the EMG, we instructed participants in the EMG study to produce moderate muscle activation (approximately 20% effort) to avoid fatigue.

We analyzed EMG data to first estimate the activation onsets of the primary muscle group of each trial, and then to determine if significant time-locked activity occurred in EMG signals from the other recorded muscles. To perform this analysis,

EMG signals from all recorded muscles were first high-pass filtered at 100 Hz (4th order zero-lag Butterworth filter), rectified, and low-pass filtered at 30 Hz (Hodges et al., 2007), and then normalized to identically processed EMG data from the maximum activation trial. EMG data were then smoothed with a 500 ms moving average. Activation onsets were defined to occur when the smoothed EMG exceeded 2 standard deviations of the EMG baseline noise with the muscle at rest. Within each participant, we then defined an EMG transient for each muscle and each trial by averaging the rectified and filtered EMG data across repeated muscle activations within a time window spanning 1 second before to 3 seconds after the activation onset of the primary muscle for the trial. To examine significant EMG magnitude changes, we performed group statistics on the maximal EMG transient for muscles of interest within each participant. To define significant temporal shifts between EMG signals, we quantified temporal shifts in each participant by determining the maximum cross-correlation between a pair of EMG transients.

fMRI Acquisition and Analysis

In 10 participants, we took measurements of brain activation associated with the voluntary muscle activation tasks (described above) using fMRI. We used a 3 Tesla (GE Signa Excite) with an 8-channel head coil. We positioned participants supine viewing a fixation crosshair, and placed foam pads to limit head motion. As in previous fMRI studies of PFM activation (Schrum et al., 2011a), we collected T2-weighted echo planar image volumes with blood oxygen level dependent (BOLD) contrast (echo time 34.5 milliseconds, flip angle 90 degrees, field of view 220 mm, pixel size 3.43 mm)

continually every 2.5 seconds during 3 imaging runs. Each volume consisted of 37 axial slices (3 mm slice thickness, 0.5 mm interslice gaps) that covered the brain from vertex to cerebellum. We additionally acquired a T1-weighted high-resolution anatomical image from each participant. We cued participants to voluntarily activate each muscle group (to approximately 20% effort) in 3 separate runs - PFM activation run, GMM activation run, and FDI activation run - as described above with the exception that participants performed additional activation blocks (6 blocks of 10 activations) in the scanner. All 10 participants performed PFM, GMM, and FDI activation runs, as well as PFM decoupling runs.

We preprocessed each participant's fMRI data using the FMRIB Expert Analysis Tool (FEAT, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/), which included skull extraction using the brain extraction tool (BET) in FSL, slice timing correction, motion correction, spatial smoothing using a Gaussian kernel with full-width half-maximum of 5 mm and nonlinear high-pass temporal filtering (100 s). We used general linear model (GLM) to examine the changes in BOLD signal associated with muscle activation for the three tasks. We performed first-level whole-brain GLM analyses of individual runs in each participant to determine the change in BOLD signal during the activation blocks compared to the rest blocks. We then performed a group-level mixed-effect (FLAME 1 in FSL) analysis, with unpaired 2-sided *t* tests, to identify voxels in standard Montreal Neurological Institute (MNI) coordinates with significant differences in response based on the muscle group being voluntary contracted by the participant. We thresheld group-level images with cluster-based correction for multiple comparisons with $Z > 2.3$ and $p < 0.05$. Since we were interested solely in sensorimotor cortical substrates of PFM muscle synergies,

results only displayed for voxels that have a greater than 0 probability of belonging to the precentral gyrus, post-central gyrus, or supplementary motor cortex according to the Harvard-Oxford cortical atlas available in FSL. We made inferences about specific Brodmann areas using the Juelich Histological Atlas.

Results

Using recordings from the PFM and GMM we found that PFM co-contracted during GMM activation (Figure 5A). Example recordings from a single participant show that during repeated activation of the GMM, participants were able to suppress PFM activity post-training (Figure 5B). However, when the participant repeatedly activated the GMM prior to the training, the PFM activated in a synchronous fashion (Figure 5A). Group data from 10 participants demonstrated that we observed this synergistic decoupling of the PFM across the study population (Figure 5C).

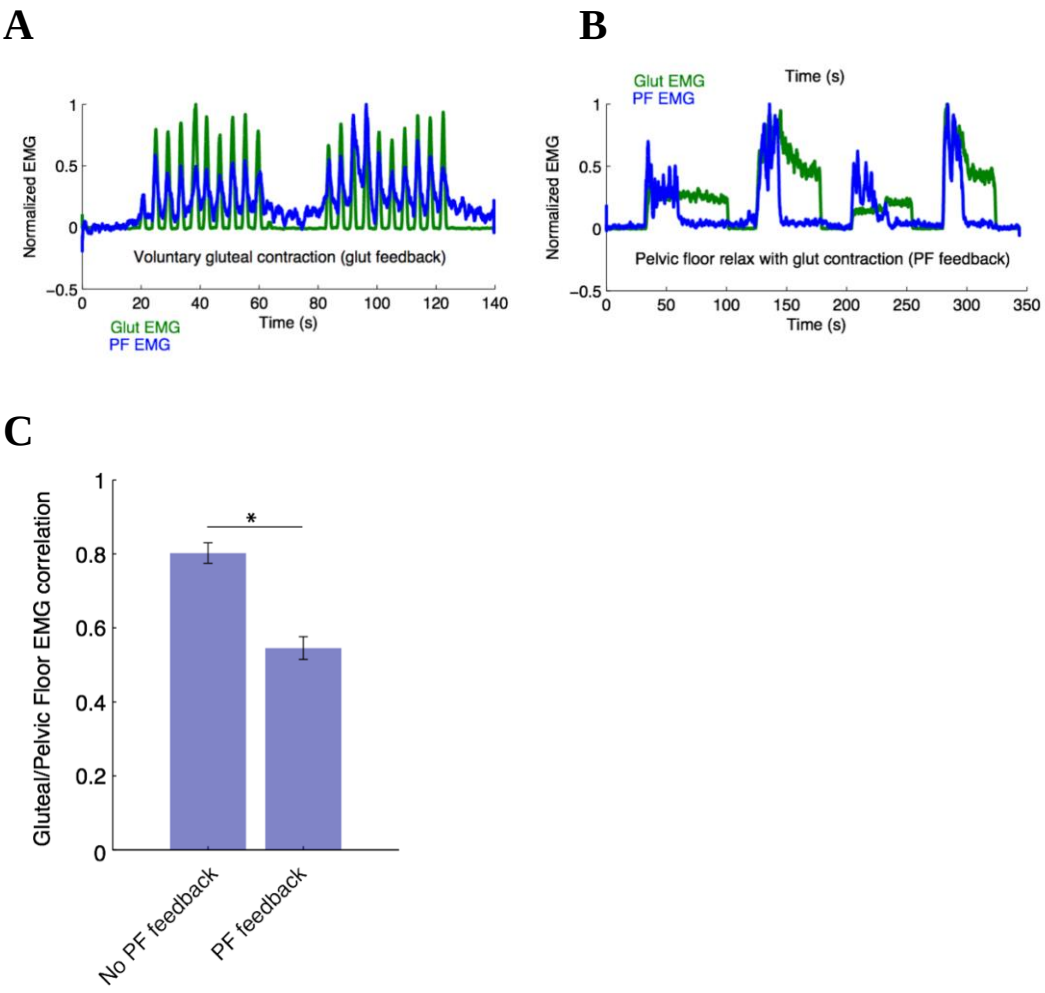

Figure 5. Electromyographic (EMG) evidence of pelvic floor muscle synergy decoupling. ***A,*** EMG signals from the pelvic floor (PFM - blue) muscles and gluteal (GMM - green) muscle, were recorded during voluntary activation of the GMM, showing inherent activity of the PFM. ***B,*** Example EMG recordings from the PFM and GMM muscles in a single participant during repeated voluntary GMM while PFM was voluntarily suppressed. Participants performed 4 blocks of 2 minute training trials. We were able to observe apparent simultaneous onset of PFM and GMM activity followed by PFM suppression after the training session. ***C,*** Statistical analysis of group data shows that GMM activity is significantly greater ($p<0.01$, **) during voluntary decoupling compared to PFM activity during voluntary GMM (inherently coupled) activation.

Using fMRI data collected while participants performed muscle activation tasks identical to those described above (Figure 5A, B), we found that a region of the medial wall of the precentral gyrus was no different during the coupled GMM activation task compared to the voluntary GMM/PFM decoupling task (Figure 6). Surprisingly, we found significant activation of the Anterior Cingulate Cortex and Left Anterior Insula during the decoupling task compared to the coupling task.

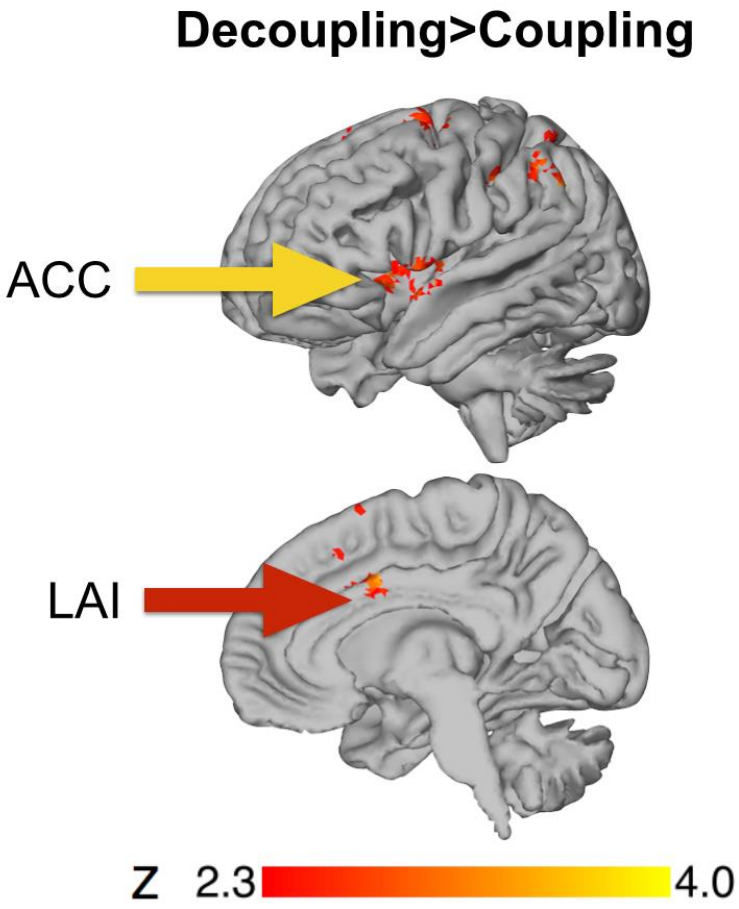

Figure 6. Functional magnetic resonance imaging (fMRI) evidence of non-motor related brain regions. Contrast of voluntary decoupling activation greater than voluntary coupling activation produced significant brain activation in the Anterior Cingulate Cortex (ACC) and Left Anterior Insula (LAI).

Discussion

Our results indicated that with appropriate training, a cortically facilitated PFM synergy can be broken, but the neural substrates that underlie this decoupling does not involve the motor cortex as originally hypothesized. Instead, execution of the decoupling task (voluntary psychomotor effort of GMM activation without PFM activity) as compared to the coupling task (GMM activation accompanied by PFM activity) results in increased activation of the anterior cingulate cortex (ACC) and left anterior insula (LAI). This is contrary to our original hypothesis that there would be a blood-oxygen-level dependent (BOLD) signal difference in the motor area associated with the PFM. This salient discovery appears to be in alignment with evidence suggesting that our decoupling task involves not only motor cortex mechanisms, but other neural substrates and autonomic nervous system (ANS) mechanisms that involve conjoint functions of the ACC and LAI - both areas involving micturition function, which inherently engages PFM activity (Di Gangi Herms et al., 2006; Medford and Critchley, 2010). This finding is consistent with former studies that have shown that pelvic floor activities are closely connected with the micturition process (Zhang et al., 2005; Seseke et al., 2006b).

Our study also demonstrated that the cortical mechanism that underlies motor control of PFM activity is inherently associated with cortical regions involved with managing continence and its associated visceral functions. Numerous studies have demonstrated consistent activation in the ACC and insula during rectal distension (Hobday et al., 2001; Lotze et al., 2001; Kern and Shaker, 2002; Verne et al., 2003), as well as the application of visceral stimulation in the upper and lower gastrointestinal tract (Derbyshire, 2003). More specifically, Seseke et al. found similar activation patterns of

the anterior insular cortex and ACC when they instructed healthy females to "contract" the PFM to mimic the interruption of voiding while feeling the urge to urinate, compared to the "relax" condition, in which they were instructed to release the PFM to mimic voiding while they felt the urge to urinate (Seseke et al., 2006a). Interestingly enough, none of their 11 participants were actually able to start voiding during the experiments, and this may implicate the involvement of the ANS. Their findings are rather consistent with our increased activation patterns of the ACC and LAI during our PFM decoupling task. Our GMM and PFM synergy decoupling task requires a significant amount of concentration on the task, cognitive awareness of PFM activation and suppression, attention to activation of the GMM, detecting error throughout the task, and interoception; all of which involve the function of the insula and ACC. The insula is a mediator of visceral sensations and is associated with interoception as it relates to sensations associated with the pelvic floor (Blok et al., 1998; Mertz et al., 2000; Kern et al., 2001; Matsuura et al., 2002; Derbyshire, 2003). We therefore believe the insula makes up a portion of the neural substrate that takes part in the cortically facilitated inhibition of the PFM while the GMM remains active. The ACC, along with the insula, has also been shown to be an important region for interoceptive awareness of such visceral sensations, and we believe this to be relevant in the case of learning about pelvic floor awareness (Critchley et al., 2004). ACC neuroimaging studies show that it is involved in cognitive processes involving attention (e.g., bladder distention) and executive control (e.g., appropriate timing of micturition) (Critchley et al., 2003; Matthews et al., 2004). Using healthy male controls and PET (positron emission Tomography) imaging, Block et al. demonstrated more cortical activity in the anterior

cingulate gyrus during micturition and during an empty bladder state, compared to urine withholding (Blok et al., 1997). We suggest that this parallels our findings of increased ACC activity during PFM relaxation (pseudo-micturition state) while the participants were instructed to feel the 'urge to urinate," compared to synergistic contraction of the PFM during the GMM activation task (pseudo- urine withholding task). Furthermore, a presumable link has been shown between urge incontinence and lesions of the forebrain such as the anterior cingulate gyrus (Andrew and Nathan, 1964; Maurice-Williams, 1974). This would support the idea that the anterior cingulate gyrus may play a specific role in functional PFM synergies, such as during the task of withholding urine. Thus, we also believe that the ACC makes up another portion of the neural substrate that takes part in the PFM decoupling task. It is apparent that our findings with the ACC and LAI play a consistent role with PFM activity as it relates to micturition, but the cortical mechanisms to explain our decoupling results warrant further discussion.

Our study also provides further cortical evidence by which motor control of the PFM may be inherently associated with the ANS. The dorsal ACC is activated when engaging in attentional or behaviorally demanding cognitive tasks (Paus, 2001). This activation is suggested to be associated with the synergy decoupling task in our experiment. From a motor function and anatomical perspective, the ACC contains cingulate motor areas that are defined by their projections into the premotor and motor cortices and spinal cord (Morecraft and Tanji, 2009). This cognitive subdivision of the ACC (Vogt et al., 1992; Devinsky et al., 1995; Bush et al., 1998; Carter et al., 1999) keeps reciprocal interconnections with the premotor and supplementary motor areas; and

has efferent paths to the autonomic, visceromotor and endocrine systems (Devinsky et al., 1995; Vogt et al., 1992).

CHAPTER FIVE

DISCUSSION

The End of Two Experiments and the Beginning of Many More

The impetus to study muscle synergies of the human pelvic floor is three-fold: 1. Pelvic floor associated disorders is prevalent in many neuro-musculoskeletal forms relevant to rehabilitation science (e.g. chronic pelvic pain, overactive bladder syndrome, incontinence, and chronic low back pain), 2. The pelvic floor serves as a feasible and well-controllable experimental paradigm to study possible mechanisms, and 3. The pelvic floor serves as a good vehicle to explore cortical and peripheral mechanisms related to understanding muscle synergies, more specifically, the muscles of the human pelvic floor. Through the scientific process of this dissertation we set a goal to understand how muscles of the pelvic floor behave in their natural healthy state; more specifically, in healthy male participants. As a result of our two original, completed experiments, we answered the questions of: 1. What are the neural substrates underlying pelvic floor muscle (PFM) synergies and 2. What cortical changes occur when these muscle synergies are broken? The specific accomplished aims were as follows: 1. The muscle synergies of the pelvic floor were cortically facilitated, and 2. We discovered that the cortical regions that are more active as a result of PFM decoupling does not involve the primary motor cortex or premotor cortex regions, but instead regions of the limbic system.

In experiment 1 (Chapter 3), we utilized electromyography (EMG), functional magnetic resonance imaging (fMRI), and transcranial magnetic stimulation (TMS) to

study pelvic floor muscle synergies in healthy male participants. In that experiment, we first demonstrated that voluntary gluteus maximus muscle (GMM) activation was accompanied by involuntary PFM activity but the reverse did not hold true. We then proceed to utilize fMRI to cortically map each individual muscle task that was performed (GMM task, PFM task, and first dorsal interossei [FDI] task). Interestingly enough, our findings suggested that voluntary PFM activation is associated with increased blood-oxygen-level dependent (BOLD) signal specifically in the region known as the supplementary motor area (SMA). Even more interesting was the association between GMM activation and increased BOLD signal in an overlapping area within SMA as well as another distinct area of the primary motor cortex. As expected, using FDI as a reference control, we further demonstrated that FDI muscle activity does not occur synergistically with the PFM and it is not associated with any overlap with the region of the SMA, which also represents motor activity of the PFM. To further validate our findings, we were able to show, utilizing TMS and T2 weighted images of each individual participant, that TMS was able to elicit motor evoked potentials (MEPs) of the PFM in the midline, where SMA was also located. More specifically with TMS, MEPs from the GMM were not produced when the TMS coil was stimulating over the area of the SMA. Having the rigor of being able to cross validate between EMG, fMRI, and TMS, we were able to demonstrate in Chapter 3 that PFM synergies are cortically facilitated.

In experiment 2 (Chapter 4), we utilized EMG and fMRI to study the cortical changes that occur when healthy male participants are trained to break the synergy found in our prior original experiment (experiment 1, Chapter 3). In this experiment, we utilized

EMG as a training session for participants to learn how to decouple the PFM synergy by training them with the use of EMG visual feedback. In our experiment, the training time sufficient for training subjects to relax the pelvic floor while GMM is contracting, was approximately 30 minutes, although participants were given 1 hour total time prior to actually performing the tasks in the fMRI scanner. Once we deemed that there was a significant difference in the coupling vs. decoupling task, participants were consented to perform the same tasks in the fMRI scanner. Contrary to our original hypothesis, we did not find differences in BOLD signal of the SMA and motor cortices. Instead, we found increased BOLD signal in the anterior cingulate cortex (ACC) and left anterior insula (LAI). We interpreted that the ACC employs a modulatory effect on the primary motor cortex and the supplementary motor area (SMA), which facilitates the suppression of cortically facilitated muscle synergies. Furthermore, our findings indicated that the left anterior insula mediates somatic and visceral attention to the interoceptive state of feeling PFM relaxation or feeling the urge to urinate. Complex motor tasks that require awareness, training, and focus on intricate somatic and visceral areas such as the pelvic floor complex may inherently require participation of the brain regions associated with interoception as well as motor control.

A possible reason why we did not demonstrate changes in the motor cortical regions may be explained by insufficient amount of time of the training period which might have resulted in our ability to produce long-lasting neuroplastic changes, as well as changes in the motor cortex or SMA. We provided our participants with a one our training that included the decoupling training with EMG feedback, as well as procedural training for the fMRI portion of the data collection. Di Gangi Hermes et al. had placed

females with stress urinary incontinence on an 11-week program of PFM training with biofeedback and found absence of activity in the supplementary motor and premotor areas, as well as a more focused activation in the primary motor (superior lateral and superior medial precentral gyrus) and somatosensory areas in the post-test condition. This might suggest that cortical plasticity may take up to 12 weeks of training to be able to demonstrate cortical changes in the motor cortex.

Conclusions and Future Directions

Human movement and bodily functions are made possible by the orchestrated interactions between the brain's neural networks and the synergistic muscles involved with the activity. This dissertation presented 2 original experiments that demonstrated the cortical mechanisms by which certain muscles of the pelvic floor are cortically facilitated, as well as how they are cortically decoupled. This process may serve as a foundational template for future muscle synergy studies.

Understanding muscle synergies is important because many disorders that are managed by rehabilitation professionals involve muscle synergies that adversely affects function and participation in meaningful activities. In our studies, we focused on muscle synergies of the human pelvic floor. Associated disorders that can benefit from understanding theses synergies are incontinence, chronic pelvic pain, and low back pain, for example. In other body regions, understanding muscle synergies would be helpful as well, such as synergies involved with neck pain and deep neck flexors; low back pain and the deep abdominal muscles; patella-femoral pain and gluteal muscles; and chronic ankle sprain and gluteal muscles. As in our experiment, we chose to study the PFM and gluteal

muscles. One logical step in understanding muscle synergies is picking relevant muscles to study. In our case, we chose the gluteus maximus muscles because of associated function, anatomical relationships, and feasibility of being able to study these muscles in the fMRI scanner. Our study was rather novel because muscle studies involving the PFM and GMM synergies are not common, yet conceptually relevant, with potential to impact pelvic floor rehabilitation, such as patients with incontinence.

Our studies involving PFM muscle synergies parallel many previous studies looking at cortical mapping of the PFM muscles (Zhang et al., 2005; Seseke et al., 2006a; Kuhtz-Buschbeck et al., 2007). To our knowledge, our studies are the first to determine that PFM are cortically facilitated and decoupling of these synergies involve increased activity in brain regions other than the motor cortex and SMA. We have shed much insight regarding the role of the GMM during PFM contractions, yet the evidence related to gluteal muscles and pelvic floor disorders is scarce. There is an abundance of literature connecting the deep abdominal muscles to the functional synergy of the PFM but not the GMM (Sapsford and Hodges, 2001; Critchley, 2002; Sapsford, 2004; Madill and McLean, 2006, 2008; Junginger et al., 2010). We believe that there is an abundance of opportunities to explore functional relationships between the GMM and PFM, especially now that we have established a clear functional synergy between the two muscle groups.

In the realm of physical therapy and rehabilitation science, motor control is a common impairment to address. The ultimate goal of a physical therapist, for example, is to retrain or restore movement through tactics such as exercise prescription. Frequently, the restoration of movement or retraining of movement requires a physical therapist's ability to reinforce a muscle synergy, build a new muscle synergy with compensatory

strategies, or decouple or break a muscle synergy that has a negative impact on the movement. For example, in a patient with patella-femoral pain, one may be excessively using his/her quadriceps muscle to perform a functional squat, while minimizing the activation and strength of the functionally synergistic gluteal muscles. Studying these two muscle synergies and how they are facilitated at a cortical level will help provide an understanding of its mechanism as to prepare for appropriate interventions. Furthermore, just as in our second study (experiment 2, Chapter 4), one would need to break the synergistic dominance of the quadriceps muscle to unload the patella-femoral elements, by increasing the demands on the gluteals. Similar to our PFM synergy studies, we were first able to determine that there is a PFM and GMM synergy, and that this synergy was cortically facilitated. This synergy decoupling was achieved through cortical facilitation of the ACC and LAI; two functional areas that involve interoception, attention, and the autonomic nervous system. Because of this, a physical therapist that is specialized in the pelvic floor may employ different exercise interventions to help facilitate more gluteal activation during pelvic floor contractions to either facilitate more PFM activity, or to suppress the PFM activity. This is just one of many examples by which understanding muscle synergies can impart more effective and novel interventions. We recommend that involve other muscles that may be synergistically involved with the PFM, or other studies that involve a completely different body region that would also benefit from understanding muscle synergies and how they can be trained to be broken.

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