Published in final edited form as:
Muscle Nerve. 2010 September ; 42(3): 363–372. doi:10.1002/mus.21694.

CAST IMMOBILIZATION INCREASES LONG-INTERVAL INTRACORTICAL INHIBITION

Brian C. Clark, PhD[1,2], **Janet L. Taylor, MD**[3], **Richard L. Hoffman, MS**[1,2], **Douglas J. Dearth, BS**[1,2], and **James S. Thomas, PT, PhD**[1,4]

[1]Institute for Neuromusculoskeletal Research, Ohio University, Athens, Ohio 45701, USA

[2]Department of Biomedical Sciences, 211 Irvine Hall, Ohio University College of Osteopathic Medicine, Athens, Ohio 45701, USA

[3]Prince of Wales Medical Research Institute and the University of New South Wales, Sydney, Australia

[4]School of Physical Therapy, Ohio University, Athens, Ohio 45701, USA

Abstract

Immobilization reduces muscle performance, and despite these performance losses being associated with neural impairments little is known regarding adaptations in cortical properties. We utilized transcranial magnetic stimulation to assess changes in flexor carpi radialis (FCR) intracortical facilitation (ICF), and short- and long-interval intracortical inhibition (SICI and LICI) in healthy humans undergoing 3 weeks of immobilization. Measurements were obtained at rest and during contraction (15% intensity). Central activation and the Hoffman reflex (H-reflex) were also assessed. Strength decreased 43.2% ± 6.1% following immobilization, and central activation also decreased (97.5% ± 2.4% to 73.2% ± 8.3%). No changes in ICF, SICI, or LICI were observed at rest; however, LICI was increased during contraction (67.5% ± 6.9% to 53.1% ± 6.7% of unconditioned response). The increase in LICI correlated with the loss of strength ($r = -0.63$). The H-reflex increased following immobilization. These findings suggest that immobilization increases intracortical inhibition during contraction, and this increase is primarily mediated by $GABA_B$ receptors.

Keywords

muscle; disuse; electromyography; motor cortex; motor evoked potential

Muscle weakness and atrophy is a common clinical phenomenon observed following cast immobilization, spaceflight, bed rest, surgery, and injury or disease. Over the past several decades the mechanisms that underpin disuse muscle atrophy have received widespread scientific attention, but much less is known regarding neurologic changes. Developing a better understanding of disuse-induced neural adaptations, and cortical adaptations in particular, is important, because neural properties play a critical role in determining muscle performance (e.g., muscle strength and reduced motor control).[1] Additionally, aging is also associated with muscle atrophy and loss of strength, and it has been suggested that disuse serves as a reasonable model of the loss of strength resulting from advancing age.[2,3] Accordingly, advancing scientific knowledge of the cortical properties altered in association

Correspondence to: B.C. Clark; clarkb2@ohio.edu.

with paradigms involving reduced muscle performance will facilitate development of effective therapeutic interventions that target restoration of muscle strength and control. This has implications for a broad spectrum of fields including geriatrics, rehabilitation medicine, neurology, and orthopedics.

It has previously been reported that immobilization (a model of disuse) results in impairment in central (neural) activation of muscle,[4,5] increases spinal excitability,[6,7] and decreases maximal motor neuron firing rate.[8] These findings indicate that immobilization induces neuroplastic changes at the level of the spinal cord and motoneurons, with the net result being an impairment in the ability of the nervous system to maximally activate skeletal muscle. At present little is known regarding the effects of immobilization on cortical properties. Transcranial magnetic stimulation (TMS) allows for a noninvasive, safe, and painless method of activating neurons of the cortex, making it possible to investigate cortical plasticity in humans.[9,10] Using single pulses of TMS to the motor cortex, we recently reported that 3 weeks of hand-wrist immobilization increases motor evoked potential (MEP) amplitude under resting conditions. During contraction, MEP amplitude does not change and the corticospinal silent period is prolonged, suggesting increased inhibition during voluntary muscle contraction.[4] Because silent period duration is mediated at both the cortical and spinal levels, we were unable to delineate whether the immobilization-induced prolongation was due to cortical or spinal adaptations.[11–13]

Intracortical facilitatory and inhibitory properties can be examined directly using paired-pulse TMS that combines a conditioning stimulus with a test stimulus at different interstimulus intervals (Fig. 1).[14–17] For example, when a subthreshold conditioning pulse (i.e., 5% less than motor threshold) precedes a suprathreshold test pulse (i.e., 30% above MT) by 15 ms, the MEP amplitude associated with the test pulse is greater than that of a single unconditioned pulse of the same intensity (intracortical facilitation, ICF). Conversely, when the interstimulus interval is 3 ms the test motor response is reduced by the conditioning pulse (short-interval intracortical inhibition, SICI), and the test motor response is also inhibited when two suprathreshold pulses are separated by 100 ms (long-interval intracortical inhibition, LICI). It is generally thought that SICI is mediated by gamma-aminobutyric acid (GABA) type A receptors ($GABA_A$),[14,18] LICI is mediated by GABA type B receptors ($GABA_B$),[18,19] and ICF is mediated by excitatory glutamatergic interneurons and N-methyl-D-aspartate (NMDA) receptors.[10,14–16] In general, SICI and ICF are mediated locally within the primary motor cortex (M1). LICI is also commonly thought to be mediated within M1,[10,20] although recent evidence suggests that it can also be influenced by spinal mechanisms.[21]

To our knowledge, only one study has used the paired-pulse TMS technique to evaluate immobilization-induced changes in intracortical facilitatory and inhibitory properties.[22] Zanette et al.[22] assessed changes in SICI and ICF following cast immobilization after wrist fracture. They evaluated ICF and SICI of the abductor pollicis brevis and the FCR under resting conditions and observed that the immobilized muscles displayed a reduction in SICI, and in the abductor pollicis there was increased ICF. However, because the aforementioned study evaluated subjects who underwent cast immobilization for a fracture it is difficult to delineate the effects of tissue injury and pain, as opposed to disuse per se, on the changes observed following immobilization. Accordingly, the purpose of this study was to determine the effect of 3 weeks of experimental wrist-hand cast immobilization on intracortical facilitatory and inhibitory properties in healthy adults. Because state-dependent adaptations (e.g., resting conditions versus during voluntary contraction) have been observed in response to both disuse and exercise interventions,[4,23] we measured SICI, LICI, and ICF at rest and during a submaximal, isometric wrist flexion contraction. We also measured the Hoffman reflex (H-reflex) to account for changes in spinal excitability associated with

immobilization. We chose the wrist flexor muscle group because distal forearm fractures are the most common type of fractures in young adults and across the lifespan,[24] and because we and others have previously observed dramatic reductions in muscle strength with an immobilization protocol of this nature.[4,25,26]

SUBJECTS AND METHODS

Ethical Approval

The Ohio University Institutional Review Board approved the study, and written informed consent was obtained. This research conformed to the standards set by the latest revision of the Declaration of Helsinki.

Subjects and Study Design

Eleven healthy subjects completed 3 weeks of wrist-hand cast immobilization (five females, six males; 20.5 ± 0.4 years; 173.9 ± 3.5 cm, 69.9 ± 4.3 kg, body mass index, BMI: 22.9 ± 0.9 kg/m^2), and nine healthy subjects served as a control group (five females, four males; 22.0 ± 1.5 years, 174.7 ± 4.7 cm, 67.8 ± 4.9 kg, BMI: 21.9 ± 0.6 kg/m^2). Subjects were excluded if they were taking any medications or supplements, had any known neurologic or orthopedic limitations, or had a BMI ≥30.0 kg/m^2. Subjects were also excluded if we were unable to observe H-reflexes under resting conditions during an orientation session. Subjects had a similar physical activity status, as potential subjects were excluded if they systematically performed resistance exercise (>1 day/week), or were classified as “very low active” or “high active” by the Lipids Research Clinics Physical Activity Questionnaire.[27] Both subject groups had the neuromuscular function of their nondominant arm tested at baseline, which included measures of wrist flexion muscle strength, central activation, resting and active motor threshold, paired-pulse TMS parameters (ICF, SICI, and LICI), and the H-reflex. Subjects were asked not to consume alcohol (abstain for 24 h) or caffeine (abstain for 4 h) prior to the testing sessions. Testing sessions were performed at the same time of day for each subject. At the conclusion of the baseline testing session the immobilization subjects were fitted with a rigid wrist-hand platform splint (immobilization of the fingers and wrist). After 21 days of immobilization and 7 days after cast removal (recovery), the aforementioned testing session was repeated. During the recovery period subjects were instructed to return to their normal daily activities, but not begin rehabilitation or a strengthening protocol. The control subjects were tested on two occasions separated by 3 weeks with no intervening activity or procedures.

Cast Immobilization Procedures

Subjects in the immobilization group were fitted with a rigid wrist-hand cast on the nondominant forearm (Model 1101–1103, Orthomerica, Orlando, Florida). The casts were made of lightweight polyethylene and extended from just below the elbow all the way past the fingers. This cast does not permit wrist flexion/extension movements nor does it permit finger usage (e.g., because the cast extends well beyond the fingers, holding a glass with the immobilized fingers is not possible). Casts were removed 2–3 times/week under supervision to wash the arm and inspect it for complications (e.g., skin lesions, edema). We ensured compliance of the casting protocol at all other times by securing athletic tape around the cast and marking the exterior layers with a custom signature stamp to allow us to tell if the subjects attempted to remove the cast. We chose the duration of the casting period based on the fact that distal forearm fractures typically require 3+ weeks of immobilization,[28] and we previously observed that 3 weeks of immobilization is long enough to dramatically reduce muscle function and alter neurophysiologic properties.[4]

Electrical and Mechanical Recordings

Electromyographic (EMG) signals were recorded from the nondominant FCR muscle using bipolar surface electrodes located longitudinally over the muscle on shaved and abraded skin with a reference electrode just distal to the medial epicondyle (Ag-AgCl, 8 mm diameter, EL258, interelectrode distance of 25 mm, Biopac Systems, Santa Barbara, California). The EMG signals were amplified (×500–1,000), bandpass-filtered (10–500 Hz), and sampled at 5,000 Hz (MP150, Biopac Systems). To quantify wrist flexion forces, subjects were seated with the elbow at 90°, the hand pronated, and the forearm supported (Biodex System 4). The wrist joint was aligned to the rotational axis of a torque motor to which a constant-length lever arm was attached (Fig. 2). The signal was scaled to maximize its resolution (208.7 mV/N·m; Biodex Researchers Tool Kit Software), smoothed over a 10-point running average, and sampled at 625 Hz (MP150 Biopac Systems). Subjects received visual feedback of all exerted forces on a 53-cm computer monitor located 1 m directly in front of them.

Muscle Strength and Central Activation

To assess maximal wrist flexion strength, subjects performed a minimum of three maximal voluntary isometric contractions (MVC) with a 1–2-min rest period between each contraction. If subjects continually recorded more force with increasing trials, or if the two highest trials were not within 5% of each other, additional trials were performed until a plateau was reached. Verbal encouragement was provided during testing. The highest value was considered the MVC.

To determine what percentage of the total force generating capacity of the wrist flexors can be produced voluntarily, a combination of voluntary and electrically stimulated contractions was performed. Transcutaneous electrical stimulation was delivered to the median nerve in the bicipital groove via stimulating electrodes (Ag-AgCl, 35 × 45 mm, No. 2015; Nikomed, Doylestown, Pennsylvania). The electrical stimuli consisted of 0.5-ms pulses (Digitimer DS7; Digitimer, Welwyn Garden City, Hertfordshire, UK). Stimuli were administered at increasing stimulation intensities until the FCR peak-to-peak (p-p) EMG amplitude reached a plateau (M_{max}), and for central activation testing the intensity was subsequently increased 20% above that eliciting M_{max}. Next, to assess central activation a supramaximal electrical doublet (100 Hz) was delivered while the subject performed a 4–5 s MVC. The increase in force immediately following the stimulation was expressed relative to a potentiated response evoked 1–2 s after the MVC, and central activation was calculated as follows: % central activation = [1 − (evoked force during MVC/evoked force following MVC)] × 100.

TMS and Intracortical Properties

TMS pulses were delivered using two connected Magstim (Wales, UK) 200^2 stimulators through a 70-mm figure-eight coil. The coil was positioned tangential to the scalp and 45° to the midline[29] so that the induced current flows in a lateral-posterior to medial-anterior direction, which predominantly activates corticospinal neurons transsynaptically.[30] The stimulation location that elicited the largest peak-to-peak (p-p) amplitude of the FCR motor evoked potential (MEP) was identified and marked on a lycra cap for coil placement.

Next, motor thresholds (MTs) were determined while subjects were seated in the dynamometer by delivering single pulses at gradually increasing stimulation intensities as we previously described.[4,31] Both resting and active MTs were determined and expressed as a percent of the maximal stimulator output. MTs were determined using a TMS intensity well below MT and gradually increasing the intensity in 2% increments until MEPs were observed. Resting MT was defined as the stimulation intensity that elicited MEPs with a p-p amplitude of ≥50 μV in at least four of eight trials. During this assessment the muscle was

completely relaxed as monitored by the EMG signal. The contraction intensity used during the active MT trials was 15% of MVC during each respective testing session. Active MT was defined as the lowest stimulation intensity that elicited MEPs with a p-p amplitude ≥2.0 times the background (interference) EMG associated with the 15% MVC contraction in at least four of eight trials. We chose to quantify the threshold in this manner rather than using an absolute voltage (e.g., 300 μV) in an attempt to minimize the influence of the nonphysiologic factors that affect the amplitude of the voluntary signal, as these are likely to differ with repeat measurements.[32]

SICI, ICF, and LICI were evaluated using paired magnetic pulses under resting conditions and during contraction. The TMS parameters for quantifying SICI, ICF, and LICI at rest and during contraction are summarized in Table 1. To quantify SICI, ICF, and LICI at rest the second (test) stimulus was set at an intensity that, when it was given alone, evoked an MEP of ≈0.5 to 1.0 mV p-p amplitude (mean stimulus intensity of 68.3 ± 10.8% of stimulator output). During contraction this stimulus intensity was set to evoke an MEP of ≈2.0 mV p-p amplitude (mean stimulus intensity of 52.3% ± 9.4% of stimulator output). Thus, stimulus intensity was adjusted across sessions to obtain the same MEP amplitude for each subject across sessions, and as such the absolute stimulus intensity was not purposely held constant across all sessions. Post-hoc analysis of our data indicated that the mean stimulus intensities did not significantly vary across session for the control or immobilization subjects ($P \geq 0.20$). For SICI and ICF quantification the intensity of the first (conditioning) stimulus was set to 95% of active MT at rest and during contraction, and for LICI quantification the first (conditioning) stimulus was set at the intensity used for the aforementioned test pulses. The interstimulus intervals for assessing SICI, ICF, and LICI were 3, 15, and 100 ms, respectively. A total of eight trials of each of these four conditions (test pulse given alone, ICF, SICI, and LICI trials) were randomly performed in blocks and averaged. The resting trials were performed first followed by the contraction trials. During the 15% MVC contractions subjects were provided with a target line and asked to exert the respective target level of force for ≈5 s with TMS pulses being delivered between 3–5 s. We chose a contraction intensity of 15% MVC, as our pilot experiments indicated that during higher intensity contractions the ability to observe intracortical inhibition was diminished. SICI, ICF, and LICI are operationally defined by expressing the mean p-p amplitude of the conditioned MEP at each interstimulus interval as a percentage of the mean p-p amplitude of the unconditioned test pulse.

H-Reflex

To determine whether immobilization induced changes in spinal excitability we measured the amplitude of the H-reflex. Single pulse electrical stimulation was delivered to the median nerve (0.5 ms duration), and the FCR H-reflex was elicited at rest and during a 15% MVC. At the start of the H-reflex assessments, recruitment curves were first developed by gradually increasing the stimulation intensity. Next, 40–60 pulses at varying intensities were delivered, and the majority of these stimuli were at an intensity surrounding maximal p-p amplitude of the H wave. The H waves were identified by criteria previously proposed,[33] and the three highest H waves observed were averaged to represent the H_{max}. We normalized the H_{max} to the M_{max} to control for potential changes in muscle cell membrane properties associated with immobilization ($[H_{max}/M_{max}] \times 100$).

Statistics

Repeated-measures analysis of variance (ANOVA) procedures followed by Sidak post-hoc tests to control for multiple comparisons were utilized to determine changes over time. Immobilization group data and control group data were analyzed separately, as they had different numbers of timepoints. The control group data were used to assess repeatability of

the experimental measures, and the immobilization group data were used to determine the time-course of adaptations to immobilization. For all analyses a two-tailed preset α-level of significance equal to 0.05 was required for statistical significance. Data are presented as mean ± standard error of the mean. Additionally, to further aid in interpretation we also report the effect size (ES; partial eta-squared). Partial eta-squared is a measure of effect size for use in ANOVA models with values ranging between 0–1.0. It represents the proportion of total variation attributable to a given factor when partialing out other factors from the total nonerror variation and is interpreted in a similar fashion as R^2 values obtained from multiple linear regression. We observed that immobilization increased LICI during contraction, and we performed simple linear regression analyses to evaluate the association between the percent changes in muscle strength and central activation in relation to LICI. The SPSS statistical package (v. 14.0, Chicago, Illinois) was used for data analysis.

RESULTS

Muscle Strength

Wrist flexion muscle strength decreased 43.2% ± 6.1% following immobilization ($P < 0.01$, ES = 0.71) and remained depressed 15.7% ± 2.0% after 1 week of recovery ($P < 0.01$, ES = 0.74) (Fig. 3). Muscle strength did not change in the control group (18.9% ± 2.6% vs. 17.9% ± 3.1 N·m; $P = 0.45$, ES = 0.07).

Central Activation

Central activation decreased from 97.5% ± 2.4% of maximum at baseline to 73.2% ± 8.3% of maximum after immobilization ($P = 0.01$, ES = 0.53) (Fig. 3). While central activation increased to 94.4% ± 2.9% of maximum after 1 week of recovery it was still significantly depressed in comparison to baseline ($P = 0.02$, ES = 0.46) (Fig. 3). Central activation did not change in the control group (93.7% ± 3.5% vs. 97.2% ± 1.2%; $P = 0.33$; ES = 0.12).

Motor Threshold

Resting MT did not change with immobilization (Baseline: 39.6% ± 1.7%, Cast Removal: 42.1% ± 2.5%, Recovery: 39.5% ± 2.1% of stimulator output; $P = 0.10$, ES = 0.24). Active MT did not change with immobilization (Baseline: 34.1% ± 1.6%, Cast Removal: 34.9% ± 1.4%, Recovery: 34.0% ± 1.6% of stimulator output; $P = 0.74$, ES = 0.03). Neither resting nor active MT changed in the control group (Resting MT: 44.3% ± 2.1% vs. 42.2% ± 2.5% of stimulator output, $P = 0.35$, ES = 0.11; Active MT: 41.2% ± 1.6% vs. 40.3% ± 2.5% of stimulator output, $P = 0.63$, ES = 0.03).

Intracortical Facilitation

ICF did not change with immobilization under resting conditions or during contraction (Rest: $P = 0.89$, ES = 0.01; Contraction: $P = 0.23$, ES = 0.14) (Fig. 4A). ICF did not change under resting conditions or during contraction in the control group (Resting ICF: 109.3% ± 10.2% vs. 124.1% ± 9.7% of test pulse, $P = 0.16$, ES = 0.23; Contraction ICF: 94.2% ± 8.3% vs. 88.9% ± 4% of test pulse, $P = 0.48$, ES = 0.02).

Short-Interval Intracortical Inhibition

SICI did not change with immobilization under resting conditions or during contraction (Rest: $P = 0.83$, ES = 0.02; Contraction: $P = 0.94$, ES = 0.01) (Fig. 4B). SICI did not change under resting conditions or during contraction in the control group (Resting SICI: 54.1% ± 19.2% vs. 61.0% ± 9.6% of test pulse, $P = 0.74$, ES = 0.02; Contraction SICI: 86.5% ± 8.3% vs. 99.9% ± 11.0% of test pulse, $P = 0.16$, ES = 0.023).

Long-Interval Intracortical Inhibition

LICI did not change with immobilization under resting conditions ($P = 0.84$, ES = 0.02) (Fig. 4C). However, LICI measured during contraction increased following immobilization ($P = 0.04$, ES = 0.32), and remained elevated after 1 week of recovery ($P = 0.02$, ES0.44) (Fig. 4C; note: a smaller value indicates the first pulse exerts a greater inhibitory effect on the second pulse, thus the interpretation is an increase in LICI). LICI did not change under resting conditions or during contraction in the control group (Resting LICI: 13.0% ± 3.1% vs. 14.1% ± 3.7% of test pulse, $P = 0.80$, ES = 0.01; Contraction LICI: 65.2% ± 13.0% vs. 63.0% ± 8.8% of test pulse, $P = 0.72$, ES = 0.02).

H-Reflex

The H-reflex increased following immobilization under resting conditions (25.0% ± 5.9% vs. 44.6% ± 7.6% H_{max}/M_{max}, $P = 0.04$, ES = 0.43) and remained elevated after 1 week of recovery (37.2% ± 7.9% H_{max}/M_{max}; $P = 0.02$, ES0.53). The H-reflex was not significantly altered following immobilization when it was recorded during a 15% MVC (41.8% ± 4.8% vs. 54.5% ± 7.3% H_{max}/M_{max}, $P = 0.07$, ES: 0.34), but it was increased after 1 week of recovery (56.8% ± 6.4% H_{max}/M_{max}, $P = 0.01$, ES = 0.65). The H-reflex did not change in the control group under resting conditions (31.1% ± 6.5% vs. 34.5% ± 8.0% H_{max}/M_{max}, $P = 0.64$, ES = 0.03) or during contraction (42.7% ± 7.0% vs. 45.9% ± 7.1% H_{max}/M_{max}, $P = 0.28$, ES = 0.14).

Association between Changes in Muscle Strength and Central Activation in Relation to LICI

The immobilization-induced increase in LICI explained 39% of the between-subject variability in the loss of muscle strength ($R^2 = 0.39$, $r = -0.63$ $P = 0.04$) (Fig. 5). The immobilization-induced increase in LICI explained 35% of the between-subject variability in the impairment in central activation ($R^2 = 0.35$, $r = -0.60$, $P = 0.05$).

Post-Hoc Control Experiment

As stated above, immobilization decreased central activation, which meant that our target contraction intensity of 15% maximal *voluntary* contraction was actually reduced following immobilization when considered relative to the muscle's maximal capacity. For example, prior to immobilization central activation was 97% of maximum, and a 15% MVC represents a contraction intensity of 14.5% of the muscle's maximal capacity. After immobilization central activation was 73% of maximum, and as such a 15% MVC represents a contraction intensity of 11% of the muscle's maximal capacity. Because LICI decreases with the level of voluntary contraction,[34] it is possible our observed increase in LICI following immobilization results from the impairment of central activation resulting in a weaker contraction when considered relative to the muscle's maximal capacity. To explore this as a potential explanation of our findings we conducted a follow-up control experiment to examine the influence of contraction intensity on LICI. Specifically, using the stimulus parameters employed in the immobilization experiment, we evaluated LICI at six different contraction intensities (5, 10, 15, 20, 25, and 40% MVC). These data were collected from a total of seven subjects (25.8 ± 2.8 years) in a single session, and the contraction intensities were randomly performed. A repeated-measures ANOVA was performed to examine the effect of contraction intensity on LICI, followed by least squares difference post-hoc tests to determine if the 15% MVC intensity differed from the other contraction intensities. We observed that LICI was graded with contraction intensity ($P = 0.01$, ES = 0.49), however, this difference was only significant at the 25% and 40% MVC intensities ($P = 0.05$ and 0.01, respectively) (Fig. 6).

DISCUSSION

This study used paired-pulse TMS to determine changes in intracortical facilitatory and inhibitory properties following 3 weeks of wrist-hand immobilization. The most novel finding of this study is that immobilization resulted in a state-dependent adaptation in LICI with an increase in LICI during contraction but no change at rest. Over the past several decades numerous experiments have been conducted to investigate changes in neuromuscular properties following prolonged periods of disuse. Most of these studies have focused on adaptations in skeletal muscle properties, such as the molecular signals of muscle atrophy.[35,36] However, it has long been suggested that "neural factors" largely mediate the loss of muscle strength and motor control associated with disuse, but despite this suggestion considerably less emphasis has been focused on the disuse-induced adaptations in neurophysiologic parameters. Our observation of an impairment in central activation following immobilization is consistent with the notion of neural factors being largely responsible for the loss of muscle strength, and it is congruent with previous work that examined the effects of both immobilization and bed rest on voluntary muscle activation.[4,5,37,38]

Most studies of the neurophysiologic mechanisms of disuse-induced muscle weakness have focused on excitability and behavioral properties of the spinal motoneurons.[7,8,39–42] This work has indicated that immobilization dramatically alters motoneuron behavior, as evidenced by a week of hand immobilization decreasing mean motor unit discharge rate 15% during a maximal voluntary contraction.[8] Several studies have used TMS to assess changes in cortical excitability following immobilization,[4,6,22,43–47] and many of these studies have reported that longer duration immobilization (i.e., 2+ weeks) increases MEP amplitude under resting conditions.[4,22,46] However, because MEP amplitude is influenced by spinal excitability it is difficult to delineate the changes in cortical versus spinal properties, as immobilization increases the H-reflex, suggesting an increase in spinal excitability.[6,7,37,39] Additionally, the increased MEP amplitude in resting muscle following immobilization has been reported to disappear during contraction, suggesting state-dependent adaptations.[4,22]

This study used paired-pulse TMS to investigate intracortical changes after immobilization. We found that immobilization produced no change in intracortical inhibition or facilitation when they were tested with the muscle at rest. ICF and SICI were also unchanged when they were tested during contraction, but LICI was increased. With an increase in spinal excitability suggested by larger H-reflexes, the increase in LICI suggests that immobilization results in altered inhibition in the motor cortex, but only during active conditions. To our knowledge, only one other study has used paired-pulse TMS to evaluate immobilization-induced changes in intracortical properties.[22] Zanette et al.[22] studied nine subjects who required 4–6 weeks of wrist-hand immobilization for a wrist fracture. They evaluated ICF and SICI of the abductor pollicis brevis and the FCR under resting conditions and observed that the immobilized muscles displayed a reduction in SICI, and that in the abductor pollicis immobilization increased ICF. Our findings are contrary to those of Zanette et al., as we observed no change in ICF or SICI under resting conditions. It is difficult to know what explains the discrepant findings, but there are several key differences between the studies. In particular, it is likely that sensory input during the immobilization differed. There was decreased muscle afferent feedback in both studies, but there was also increased pain when immobilization was required following a fracture. In addition, the duration of immobilization was longer in the study of Zanette et al., and slightly different intensities were used for the conditioning pulses, although the levels of SICI and ICF in control conditions were similar in the two studies. It is also possible that the discrepancy may be due to the different muscle groups studied, possible swelling when the limb was first

injured, or the slight amount of wrist and finger movement that was permitted in our study when the cast was removed 2–3 times per week to evaluate the limb for skin complications and to allow for cleansing.

Our finding of an increase in LICI during contraction is consistent with our previous observation of immobilization prolonging the silent period,[4] as they both reflect increased levels of inhibition. In contrast, SICI was not altered by immobilization. SICI and LICI are thought to be mediated through different GABA receptors[14,18] and can show different behavior in response to afferent input[48] as well as in interactions with other forms of cortical inhibition.[18,49] Thus, the increase in LICI suggests an increase in inhibitory actions through $GABA_B$ receptors. A possible confounding mechanism has been ruled out by our post-hoc control experiment. Because LICI decreases with the level of voluntary contraction,[34] our observed increase in LICI following immobilization may have been due to immobilization-induced impairments in central activation, which would result in an overall weaker target contraction when considered relative to the muscle's maximal capacity. However, we observed no differences in LICI for contraction intensities between 5%–20% MVC, which suggests that the immobilization-induced increase in LICI was not likely driven by potential changes in contraction intensity.

The findings of decreased maximal voluntary force and activation and increased LICI raise the question of whether the mechanisms underlying LICI play a functional role in determining muscle strength/weakness. Additionally, many of the changes that accompany the loss of strength with disuse coincide with those observed with aging,[3] and recent preliminary data from our laboratory indicates that older adults exhibit higher levels of LICI in comparison to young adults.[50] Therefore, we examined the association between the increase in LICI and the loss of muscle strength in this study and observed that 39% of the between-subject variability in the loss of muscle strength was explained by the increase in LICI. We found a similar relationship between LICI and central activation following immobilization. So, while a correlation between two variables does not necessarily indicate causality, these observations suggest that LICI may be associated with muscle strength/weakness. Future studies that incorporate experimental manipulations to selectively target maintenance of muscle strength during immobilization will be needed to more directly examine the mechanistic influence of LICI on muscle performance. Additionally, future work is needed to determine if these findings can be extended to other models/paradigms that present with muscle weakness and atrophy such as aging, spinal cord injury, and bed rest.

There are several limitations of this study that should be noted. The first relates to the intensity of the conditioning pulses. For the measurement of ICF and SICI we based the intensity of the conditioning pulse on the active MT. While this is a common approach, the threshold for inhibition and facilitation may not necessarily be related to the MT. However, it is unlikely this is a major confound, as active MT did not change following immobilization. It is also possible that we would have observed different findings if we had used a lower conditioning pulse. For example, intracortical excitability reflects a balance between activation of the SICI and ICF systems, but that at lower conditioning pulse intensities (e.g., 70% active MT) SICI can be examined independently of the effects on ICF.[51] Accordingly, it is plausible that a potential change in SICI may have been masked by our high conditioning pulse intensity (95% AMT). Similarly, our subjects exhibited high levels of resting LICI during the preimmobilization testing session, and as such it is possible that the magnitude of inhibition is near maximum. It may thus be difficult to detect an even further degree of inhibition following immobilization. Another limitation relates to delimiting our findings to immobilization paradigms and disuse periods of relatively short durations. For example, it is unclear whether similar observations would be made with a

different disuse paradigm (e.g., bed rest, spaceflight), and it is probable that the relative contribution of neural and muscular factors on the loss of strength dynamically changes over time, with the muscular component contributing more as the duration of disuse is prolonged.[52]

In summary, we evaluated changes in intracortical facilitatory and inhibitory properties, H-reflex excitability, and central activation before, after, and during the recovery from 3 weeks of wrist-hand immobilization. We observed that immobilization decreased muscle strength, and much of this induced weakness was due to impairments in central neural activation of the wrist flexors. We also observed that immobilization increased the H-reflex and resulted in a state-dependent adaptation in LICI with an increase in LICI during contraction but no change at rest. Additionally, we observed that the immobilization-induced loss of strength was correlated with an increase in LICI. In contrast, SICI was not altered by immobilization. When these findings are collectively considered, they suggest that immobilization increases intracortical inhibition during contraction and that this increase is primarily mediated through $GABA_B$ receptors. These findings also underscore the importance of the nervous system in determining muscle performance, and suggest that clinical interventions designed to attenuate the negative effects of disuse should target both the nervous and muscular systems.

Abbreviations

BMI	body mass index
EMG	electromyography
ES	partial eta-squared effect size
FCR	flexor carpi radialis
GABA	gamma-aminobutyric acid
H_{max}	maximum amplitude of the Hoffman reflex
H-reflex	Hoffman reflex
ICF	intracortical facilitation
LICI	long-interval intracortical inhibition
M1	primary motor cortex
MEP	motor-evoked potential
M_{max}	maximal compound muscle fiber action potential
MT	motor threshold
MVC	maximal voluntary contraction
NMDA	N-methyl-D-aspartate
p-p	peak-to-peak
SICI	short-interval intracortical inhibition
TMS	transcranial magnetic stimulation

REFERENCES

1. Clark BC. In vivo alterations in skeletal muscle form and function following disuse atrophy. Med Sci Sports Exerc. 2009; 41:1869–1875. [PubMed: 19727027]
2. Bortz WM 2nd. Disuse and aging. JAMA. 1982; 248:1203–1208. [PubMed: 7109139]

3. Timiras PS. Disuse and aging: same problem, different outcomes. J Gravit Physiol. 1994; 1:P5–P7. [PubMed: 11538760]
4. Clark B, Issac LC, Lane JL, Damron LA, Hoffman RL. Neuromuscular plasticity during and following 3-weeks of human forearm cast immobilization. J Appl Physiol. 2008; 105:868–878. [PubMed: 18635877]
5. Kawakami Y, Akima H, Kubo K, Muraoka Y, Hasegawa H, Kouzaki M, et al. Changes in muscle size, architecture, and neural activation after 20 days of bed rest with and without resistance exercise. Eur J Appl Physiol. 2001; 84:7–12. [PubMed: 11394257]
6. Lundbye-Jensen J, Nielsen JB. Central nervous adaptations following 1 wk of wrist and hand immobilization. J Appl Physiol. 2008; 105:139–151. [PubMed: 18450985]
7. Lundbye-Jensen J, Nielsen JB. Immobilization induces changes in presynaptic control of group Ia afferents in healthy humans. J Physiol. 2008; 586:4121–4135. [PubMed: 18599534]
8. Seki K, Kizuka T, Yamada H. Reduction in maximal firing rate of motoneurons after 1-week immobilization of finger muscle in human subjects. J Electromyogr Kinesiol. 2007; 17:113–120. [PubMed: 16448820]
9. Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. Lancet Neurol. 2003; 2:145–156. [PubMed: 12849236]
10. Reis J, Swayne OB, Vandermeeren Y, Camus M, Dimyan MA, Harris-Love M, et al. Contribution of transcranial magnetic stimulation to the understanding of cortical mechanisms involved in motor control. J Physiol. 2008; 586:325–351. [PubMed: 17974592]
11. Chen R, Lozano AM, Ashby P. Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. Exp Brain Res. 1999; 128:539–542. [PubMed: 10541749]
12. Wilson SA, Lockwood RJ, Thickbroom GW, Mastaglia FL. The muscle silent period following transcranial magnetic cortical stimulation. J Neurol Sci. 1993; 114:216–222. [PubMed: 8445404]
13. Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W. Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. Ann Neurol. 1996; 40:367–378. [PubMed: 8797526]
14. Ziemann U. Pharmacology of TMS. Suppl Clin Neurophysiol. 2003; 56:226–231. [PubMed: 14677399]
15. Ziemann U. TMS and drugs. Clin Neurophysiol. 2004; 115:1717–1729. [PubMed: 15261850]
16. Ziemann U, Lonnecker S, Paulus W. Inhibition of human motor cortex by ethanol. A transcranial magnetic stimulation study. Brain. 1995; 118(Pt 6):1437–1446. [PubMed: 8595475]
17. Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W. The effect of lorazepam on the motor cortical excitability in man. Exp Brain Res. 1996; 109:127–135. [PubMed: 8740215]
18. Florian J, Muller-Dahlhaus M, Liu Y, Ziemann U. Inhibitory circuits and the nature of their interactions in the human motor cortex a pharmacological TMS study. J Physiol. 2008; 586:495–514. [PubMed: 17991698]
19. McDonnell MN, Orekhov Y, Ziemann U. The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. Exp Brain Res. 2006; 173:86–93. [PubMed: 16489434]
20. Nakamura H, Kitagawa H, Kawaguchi Y, Tsuji H. Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. J Physiol. 1997; 498(Pt 3):817–823. [PubMed: 9051592]
21. McNeil CJ, Martin PG, Gandevia SC, Taylor JL. The response to paired motor cortical stimuli is abolished at a spinal level during human muscle fatigue. J Physiol. 2009; 587:5601–5612. [PubMed: 19805743]
22. Zanette G, Manganotti P, Fiaschi A, Tamburin S. Modulation of motor cortex excitability after upper limb immobilization. Clin Neurophysiol. 2004; 115:1264–1275. [PubMed: 15134693]
23. Aagaard P, Simonsen EB, Andersen JL, Magnusson P, Dyhre-Poulsen P. Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. J Appl Physiol. 2002; 92:2309–2318. [PubMed: 12015341]
24. WHO. WHO. , editor. The burden of musculoskeletal conditions at the start of the new millenium; WHO Technical Report Series. Volume World Health Organization Technical Report Series. 2003. p. 1-218.

25. Clark BC, Manini TM, Hoffman RL, Russ DW. Restoration of voluntary muscle strength following 3-weeks of cast immobilization is suppressed in women compared to men. Arch Phys Med Rehabil. 2009; 90:178–180. [PubMed: 19154845]
26. Miles MP, Clarkson PM, Bean M, Ambach K, Mulroy J, Vincent K. Muscle function at the wrist following 9 d of immobilization and suspension. Med Sci Sports Exerc. 1994; 26:615–623. [PubMed: 8007811]
27. Ainsworth BE, Jacobs DR Jr, Leon AS. Validity and reliability of self-reported physical activity status: the Lipid Research Clinics questionnaire. Med Sci Sports Exerc. 1993; 25:92–98. [PubMed: 8423761]
28. McKeag, DB.; Moeller, JL. ACSM's Primary Care Sports Medicine. 2nd Edition. Lippincott Williams and Wilkins; 2007.
29. Brasil-Neto JP, Cohen LG, Panizza M, Nilsson J, Roth BJ, Hallett M. Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. J Clin Neurophysiol. 1992; 9:132–136. [PubMed: 1552001]
30. Werhahn KJ, Fong JK, Meyer BU, Priori A, Rothwell JC, Day BL, et al. The effect of magnetic coil orientation on the latency of surface EMG and single motor unit responses in the first dorsal interosseous muscle. Electroencephalogr Clin Neurophysiol. 1994; 93:138–146. [PubMed: 7512920]
31. Farina D, Merletti R, Enoka RM. The extraction of neural strategies from the surface EMG. J Appl Physiol. 2004; 96:1486–1495. [PubMed: 15016793]
32. Damron LA, Hoffman RL, Dearth DJ, Clark BC. Quantification of the corticospinal silent period evoked via transcranial magnetic brain stimulation. J Neuro Sci Methods. 2008; 173:121–128.
33. Miller TA, Mogyoros I, Burke D. Homonymous and heteronymous monosynaptic reflexes in biceps brachii. Muscle Nerve. 1995; 18:585–592. [PubMed: 7753120]
34. Hammond G, Vallence AM. Modulation of long-interval intracortical inhibition and the silent period by voluntary contraction. Brain Res. 2007; 1158:63–70. [PubMed: 17559815]
35. Jackman RW, Kandarian SC. The molecular basis of skeletal muscle atrophy. Am J Physiol Cell Physiol. 2004; 287:C834–C843. [PubMed: 15355854]
36. Zhang P, Chen X, Fan M. Signaling mechanisms involved in disuse muscle atrophy. Med Hypotheses. 2007; 69:310–321. [PubMed: 17376604]
37. Duchateau J. Bed rest induces neural and contractile adaptations in triceps surae. Med Sci Sports Exerc. 1995; 27:1581–1589. [PubMed: 8614311]
38. Duchateau J, Hainaut K. Electrical and mechanical changes in immobilized human muscle. J Appl Physiol. 1987; 62:2168–2173. [PubMed: 3610913]
39. Clark BC, Manini TM, Bolanowski SJ, Ploutz-Snyder LL. Adaptations in human neuromuscular function following prolonged unweighting: II. Neurological properties and motor imagery efficacy. J Appl Physiol. 2006; 101:264–272. [PubMed: 16514003]
40. Clark BC, Pierce JR, Manini TM, Ploutz-Snyder LL. Effect of prolonged unweighting of human skeletal muscle on neuromotor force control. Eur J Appl Physiol. 2007; 100:53–62. [PubMed: 17287986]
41. Seki K, Taniguchi Y, Narusawa M. Effects of joint immobilization on firing rate modulation of human motor units. J Physiol. 2001; 530:507–519. [PubMed: 11158280]
42. Stevens JE, Pathare NC, Tillman SM, Scarborough MT, Gibbs CP, Shah P, et al. Relative contributions of muscle activation and muscle size to plantarflexor torque during rehabilitation after immobilization. J Orthop Res. 2006; 24:1729–1736. [PubMed: 16779833]
43. Facchini S, Romani M, Tinazzi M, Aglioti SM. Time-related changes of excitability of the human motor system contingent upon immobilisation of the ring and little fingers. Clin Neurophysiol. 2002; 113:367–375. [PubMed: 11897537]
44. Huber R, Ghilardi MF, Massimini M, Ferrarelli F, Riedner BA, Peterson MJ, et al. Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. Nat Neurosci. 2006; 9:1169–1176. [PubMed: 16936722]
45. Kaneko F, Murakami T, Onari K, Kurumadani H, Kawaguchi K. Decreased cortical excitability during motor imagery after disuse of an upper limb in humans. Clin Neurophysiol. 2003; 114:2397–2403. [PubMed: 14652100]

46. Roberts DR, Ricci R, Funke FW, Ramsey P, Kelley W, Carroll JS, et al. Lower limb immobilization is associated with increased corticospinal excitability. Exp Brain Res. 2007; 181:213–220. [PubMed: 17361426]

47. Zanette G, Tinazzi M, Bonato C, di Summa A, Manganotti P, Polo A, et al. Reversible changes of motor cortical outputs following immobilization of the upper limb. Electroencephalogr Clin Neurophysiol. 1997; 105:269–279. [PubMed: 9284234]

48. Rosenkranz K, Rothwell JC. Differential effect of muscle vibration on intracortical inhibitory circuits in humans. J Physiol. 2003; 551:649–660. [PubMed: 12821723]

49. Chen R. Interactions between inhibitory and excitatory circuits in the human motor cortex. Exp Brain Res. 2004; 154:1–10. [PubMed: 14579004]

50. McGinley M, Hoffman RL, Russ DW, Thomas JS, Clark BC. Older adults exhibit more intracortical inhibition and less intracortical facilitation than young adults. J Am Osteopath Assoc. (in press).

51. Ortu E, Deriu F, Suppa A, Tolu E, Rothwell JC. Effects of volitional contraction on intracortical inhibition and facilitation in the human motor cortex. J Physiol. 2008; 586:5147–5159. [PubMed: 18787036]

52. de Boer MD, Maganaris CN, Seynnes OR, Rennie MJ, Narici MV. Time course of muscular, neural and tendinous adaptations to 23 day unilateral lower-limb suspension in young men. J Physiol. 2007; 583:1079–1091. [PubMed: 17656438]

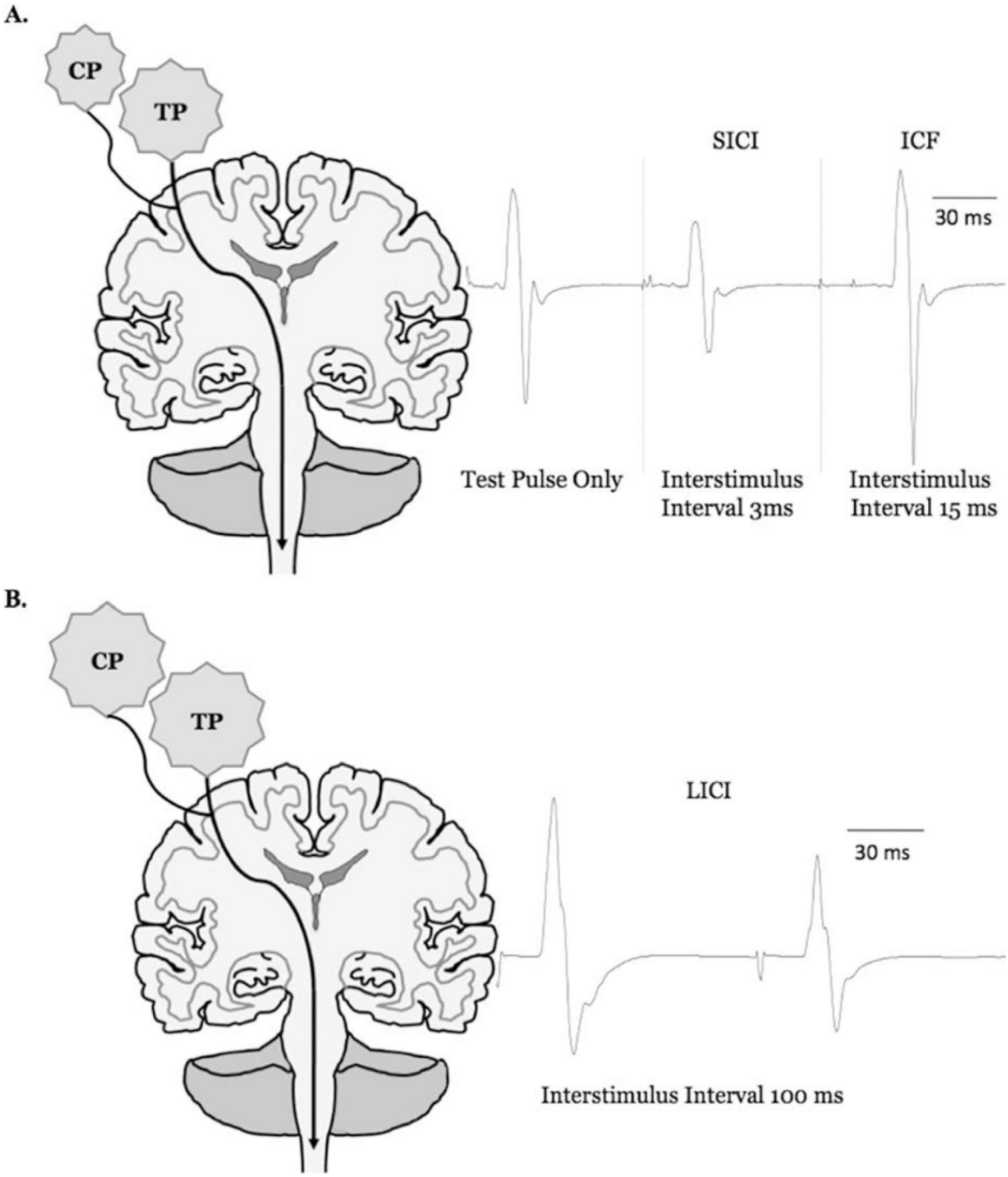

FIGURE 1.

The change of MEP sizes obtained with paired pulse TMS. (**A**) Measurement of SICI and ICF. The intensity of the conditioning pulse (CP) was set 5% below active motor threshold, and the test pulse (TP) was set to evoke MEPs between 0.5–1 mV. At short interstimulus intervals (e.g., 3 ms) the CP inhibits the MEP in comparison to the TP only (SICI), whereas at longer interstimulus intervals (e.g., 15 ms) it facilitates the MEP (ICF). (**B**) Measurement of LICI. To quantify LICI, two pulses of the same size were delivered at an interstimulus interval of 100 ms. This results in the second MEP being inhibited in comparison to the first MEP.

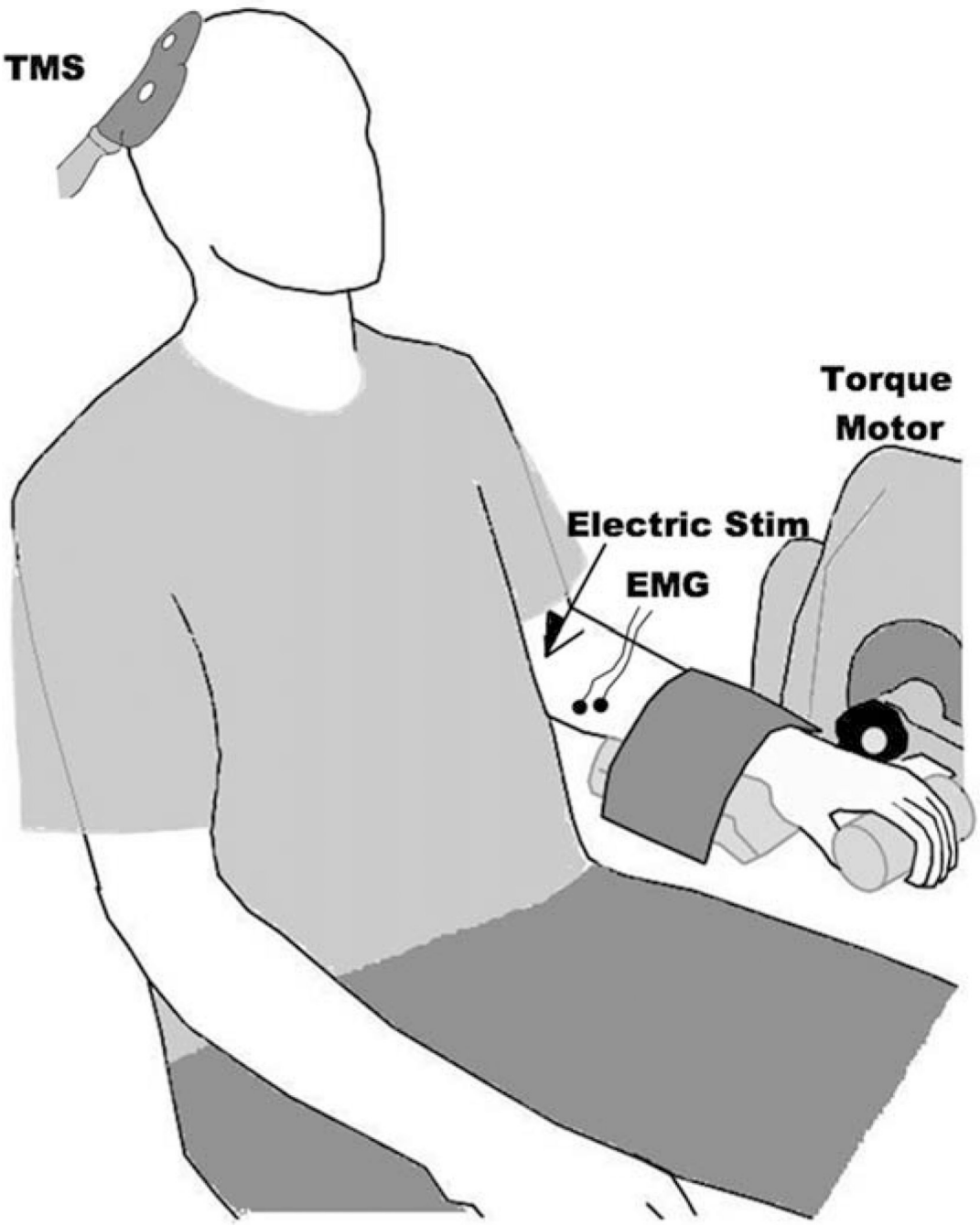

FIGURE 2.

The experimental setup for recording mechanical forces and electrical signals from the wrist flexor muscles. EMG was recorded from the flexor carpi radialis muscle, and torque was recorded from the wrist flexors. Paired-pulse TMS was performed to evaluate intracortical properties, and electrical stimulation of the median nerve was performed to evaluate central activation.

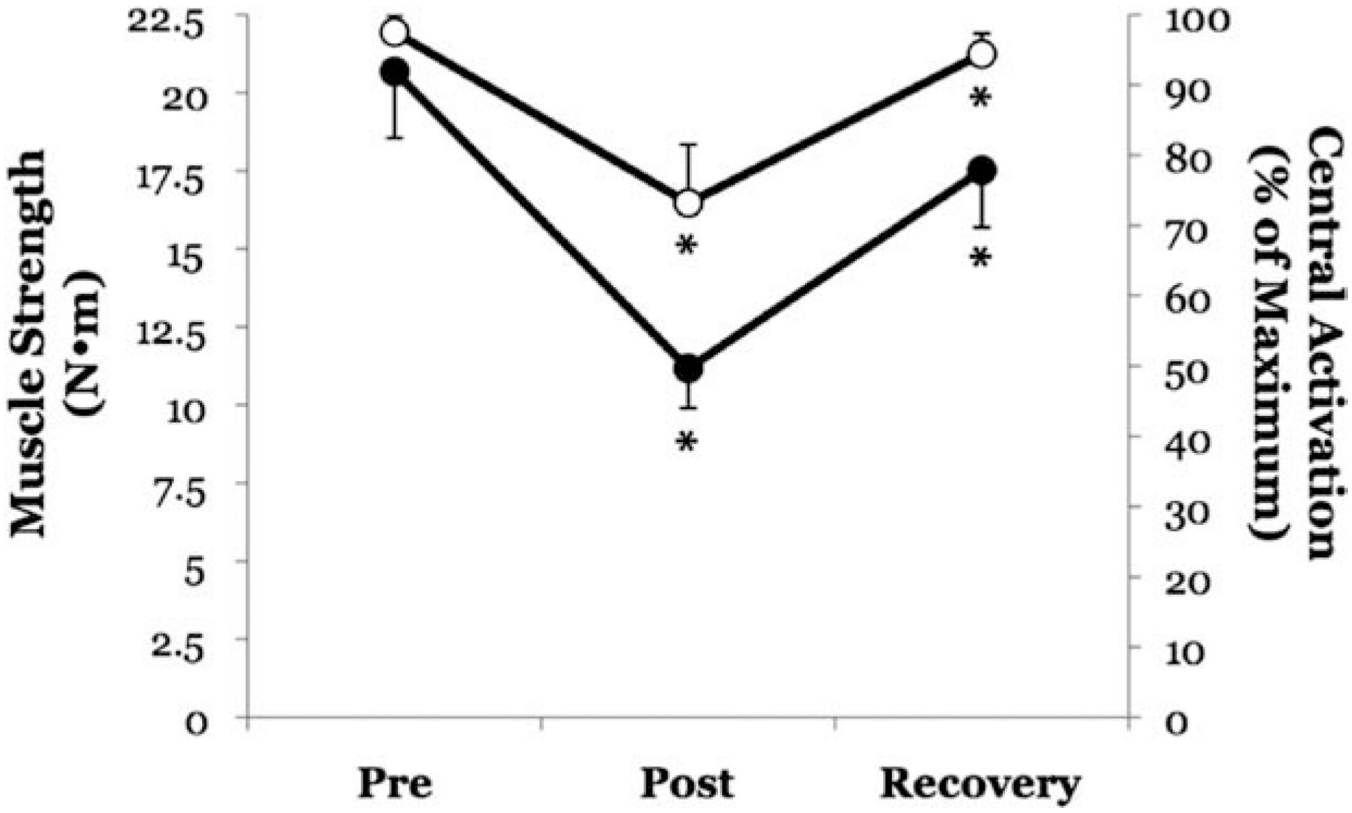

FIGURE 3.

Immobilization decreased muscle strength (closed circles; left axis) and central activation (open circles; right axis). Wrist flexion muscle strength decreased ~43% following immobilization, and strength remained ~15% below baseline levels after 1 week of recovery. No changes in muscle strength or central activation were observed in the control group. Central activation decreased from ~98% of maximum before immobilization to ~73% following immobilization. After 1 week of recovery, central activation was ~94% of maximum, but this was still less than was observed at baseline. *Significantly different from preimmobilization (baseline) value ($P \leq 0.05$).

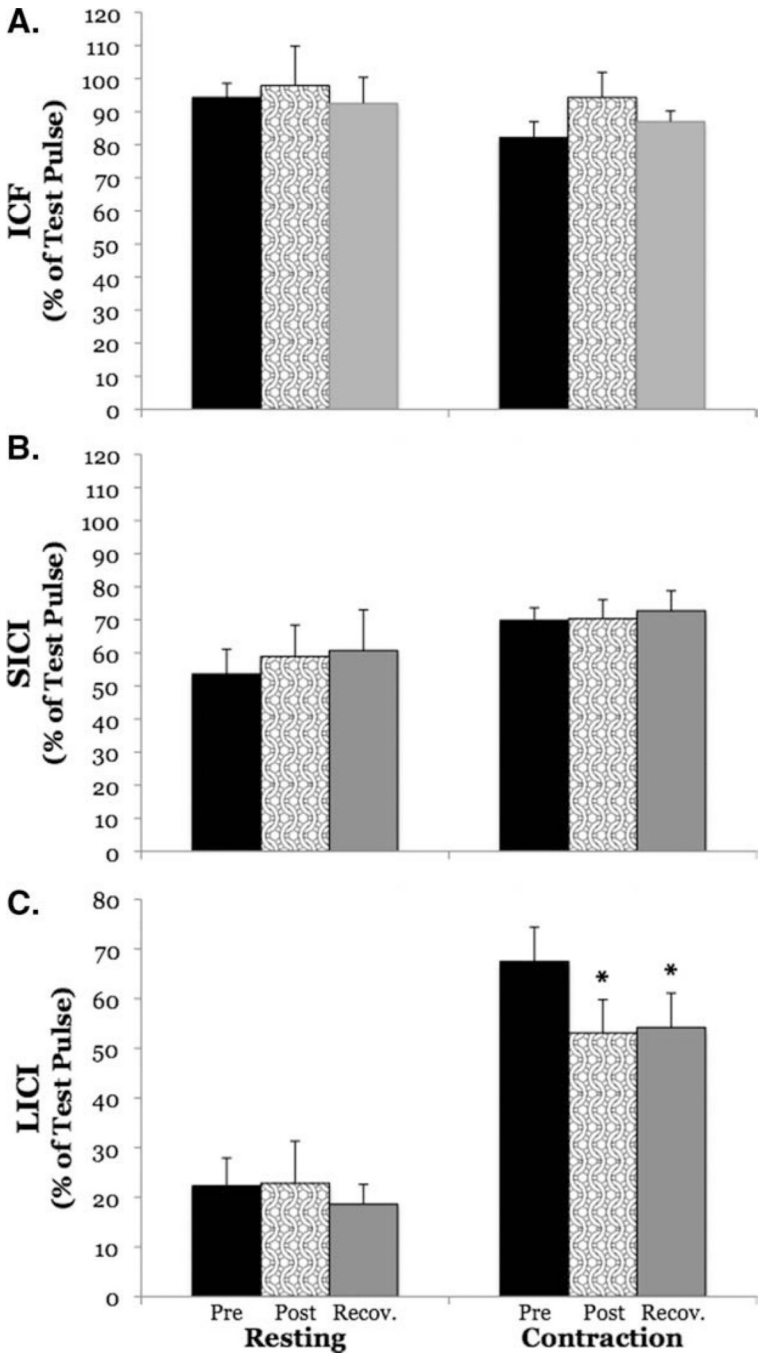

FIGURE 4.

Immobilization increased LICI. (**A**) ICF recorded under resting conditions and during a 15% MVC contraction did not change with immobilization. (**B**) SICI recorded under resting conditions and during a 15% MVC contraction did not change with immobilization. (**C**) LICI recorded under resting conditions did not change with immobilization. However, LICI recorded during a 15% MVC contraction increased following immobilization, and remained elevated after 1 week of recovery (note: a smaller value indicates the first pulse exerts a greater inhibitory effect on the second pulse; thus, the interpretation is an increase in LICI). *Significantly different from preimmobilization (baseline) value ($P \leq 0.05$).

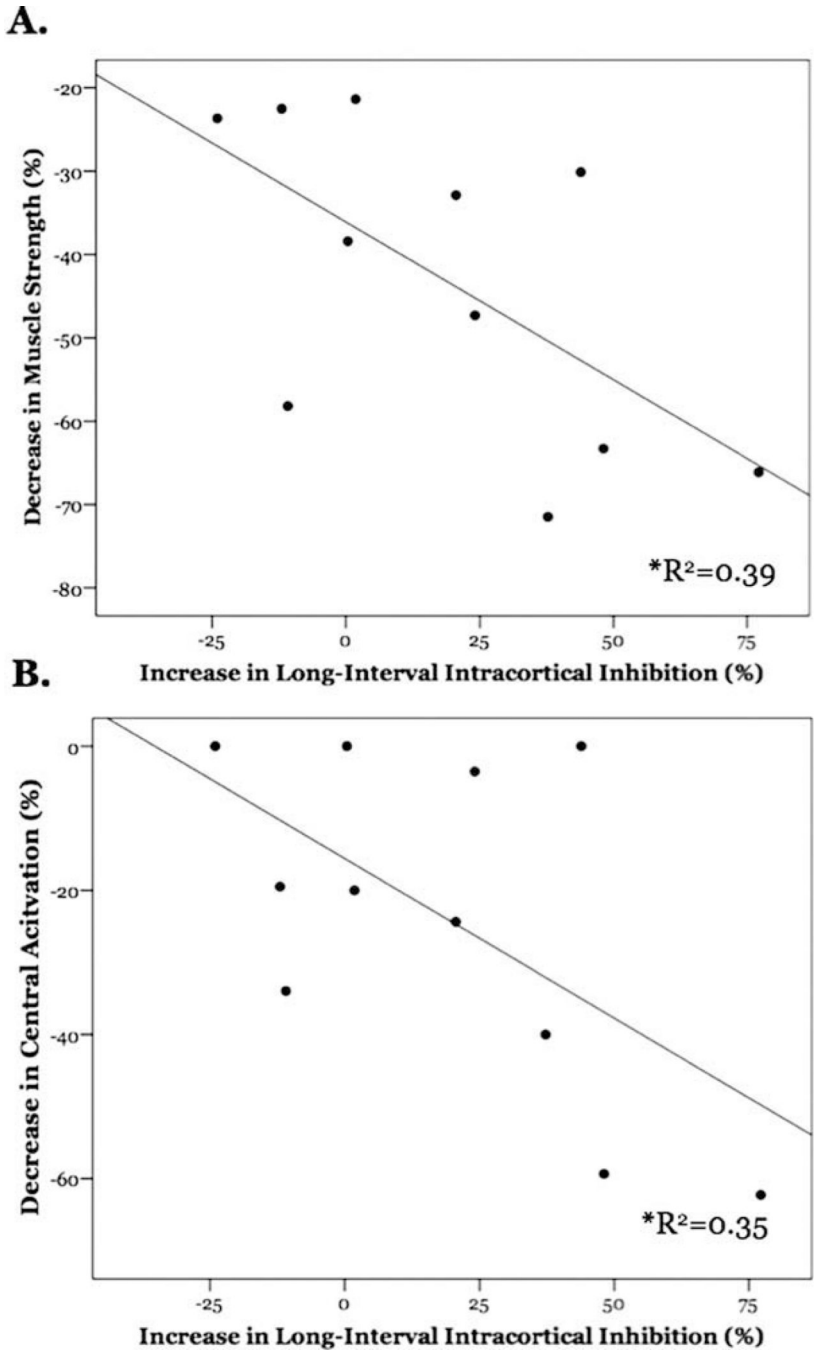

FIGURE 5.

The immobilization-induced increase in LICI explained 39% of the between-subject variability in the loss of muscle strength (**A**) and 35% of the between-subject variability in the impairment in central activation (**B**). **P* ≤ 0.05.

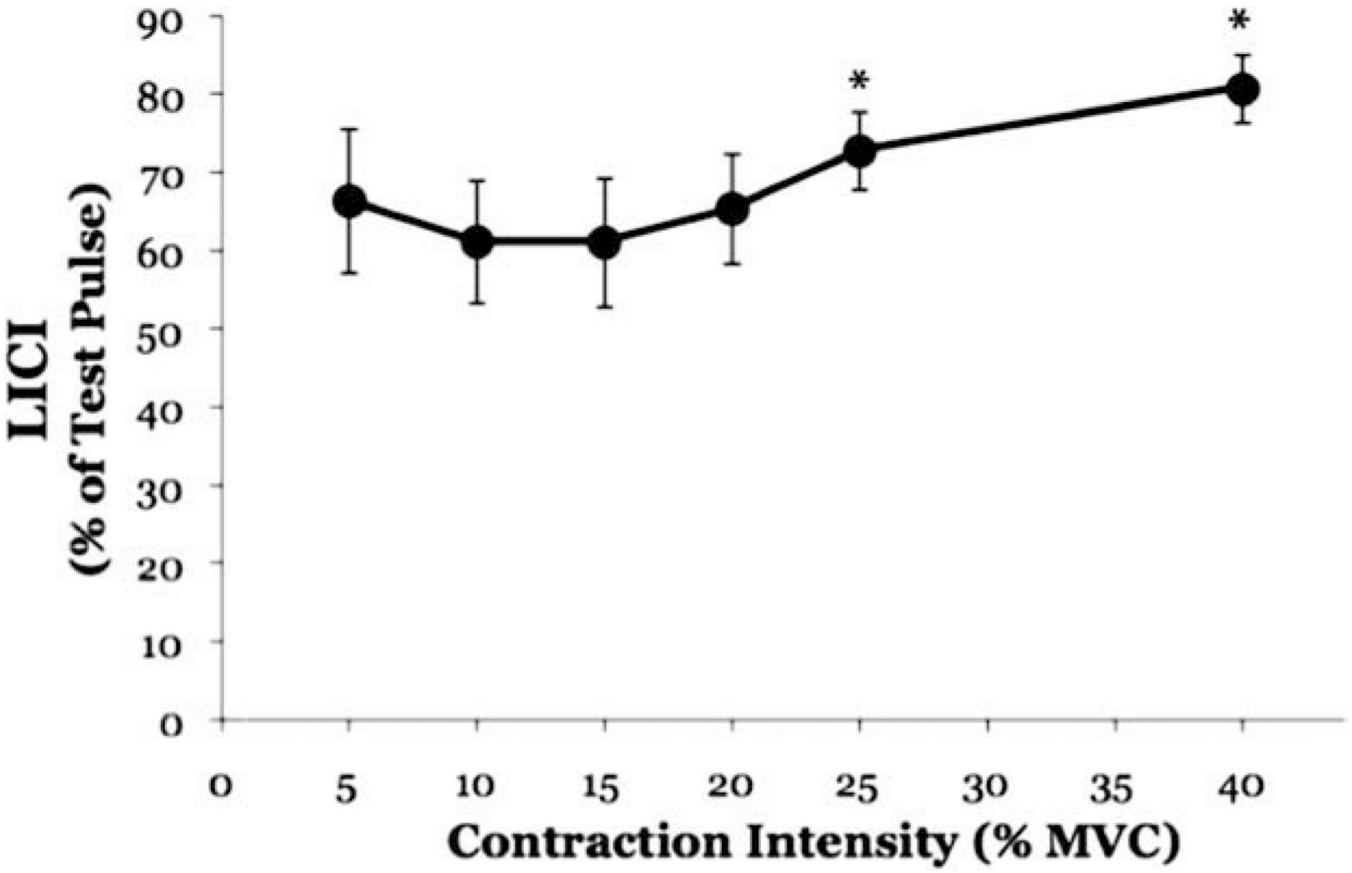

FIGURE 6.

Change in LICI in the flexor carpi radialis muscle at different contraction intensities. A control experiment was conducted to examine the relationship between the change in LICI and contraction intensity. LICI was graded with contraction intensity, but this difference was only significant above 25% MVC. *Significantly different from 15% MVC ($P \leq 0.05$).

Table 1

Summary of stimulus parameters associated with determining short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and long-interval intracortical inhibition (LICI) at rest and during contraction.

	SICI	ICF	LICI
Resting condition			
Conditioning pulse intensity	0.95 active MT	0.95 active MT	Same as TP intensity at rest
Test pulse intensity	SI evoking a 0.5–1 mV MEP	SI evoking a 0.5–1 mV MEP	SI evoking a 0.5–1 mV MEP
Interstimulus interval	3 ms	15 ms	100 ms
Contraction condition			
Conditioning pulse intensity	0.95 active MT	0.95 active MT	Same as TP intensity during contraction
Test pulse intensity	SI evoking a 2.0 mV MEP	SI evoking a 2.0 mV MEP	SI evoking a 2.0 mV MEP
Interstimulus interval	3 ms	15 ms	100 ms

TP, test pulse; MT, motor threshold; SI, stimulus intensity.