Motor Cortex Reorganization and Impaired Function in the Transition to Sustained Muscle Pain

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Abstract

Primary motor cortical (M1) adaptation has not been investigated in the transition to sustained muscle pain. Daily injection of nerve growth factor (NGF) induces hyperalgesia reminiscent of musculoskeletal pain and provides a novel model to study M1 in response to progressively developing muscle soreness. Twelve healthy individuals were injected with NGF into right extensor carpi radialis brevis (ECRB) on Days 0 and 2 and with hypertonic saline on Day 4. Quantitative sensory and motor testing and assessment of M1 organization and function using transcranial magnetic stimulation were performed prior to injection on Days 0, 2, and 4 and again on Day 14. Pain and disability increased at Day 2 and increased further at Day 4. Reorganization of M1 was evident at Day 4 and was characterized by increased map excitability. These changes were accompanied by reduced intracortical inhibition and increased intracortical facilitation. Interhemispheric inhibition was reduced from the "affected" to the "unaffected" hemisphere on Day 4, and this was associated with increased pressure sensitivity in left ECRB. These data provide the first evidence of M1 adaptation in the transition to sustained muscle pain and have relevance for the development of therapies that seek to target M1 in musculoskeletal pain.

Key words: elbow pain, interhemispheric inhibition, nerve growth factor, primary motor cortex, transcranial magnetic stimulation

Introduction

Motor dysfunction is a prominent feature of chronic musculoskeletal pain. Yet, the neural mechanisms that mediate this effect are poorly understood. Although cross-sectional studies reveal altered organization and function of the primary motor cortex (M1) in the chronic stage of pain, and these changes are associated with pain severity and motor dysfunction, there has been no longitudinal investigation of M1 in the transition from acute to sustained pain.

Multiple aspects of M1 organization and function are distorted when musculoskeletal pain persists. Motor cortical representations show greater overlap and a reduced number of discrete peaks in chronic elbow (Schabrun, Hodges et al. 2014) and low back pain (Tsao et al. 2008; Tsao et al. 2011; Schabrun, Elgueta-Cancino et al. 2014), whereas activity in inhibitory networks (Γ-aminobutyric acid [GABA] mediated) is reduced (Masse-Alarie et al. 2012; Schwenkreis et al. 2003). These adaptations are associated with pain severity (Schwenkreis et al. 2003; Schabrun, Elgueta-Cancino et al. 2014; Schabrun, Hodges et al. 2014) and/or motor dysfunction (Tsao et al. 2008). In addition, a release of inhibition from the "affected" (corresponding to the painful region) to the "unaffected" M1, known as interhemispheric inhibition (IHI), may underpin observations of bilateral motor dysfunction in unilateral pain conditions (Pienimaki et al. 1997; Bisset et al. 2006; Alizadehkhaiyat et al. 2007).
these findings, M1 adaptation in the transition to sustained muscle pain has not been characterized. It is unknown how M1 is altered during the transition to sustained pain and at what time-point changes develop. In particular, the amount of pain (severity and/or duration) required to trigger M1 adaptation has not been examined.

Repeated injection of nerve growth factor (NGF) has been shown to sensitize peripheral and central neuronal mechanisms and is a novel model capable of inducing progressive muscle soreness, mechanical hyperalgesia, and temporal summation of pressure pain that can last up to 14 days (Hayashi et al. 2013). NGF-induced muscle pain mimics the time-course (slowly developing muscle soreness) and processes involved in the transition to chronic musculoskeletal pain (Hayashi et al. 2013) and thus provides a realistic model to investigate M1 adaptation in the transition to sustained pain. Moreover, acute muscle pain induced by injecting hypertonic saline in a system already sensitized by NGF provides a unique opportunity to evaluate M1 in response to a clinically relevant, acute exacerbation of muscle pain.

In the present study, the NGF model was used to 1) investigate the nature and time-course of M1 organization (representational maps) and function (intracortical networks and interhemispheric networks) in response to progressively developing muscle soreness, 2) determine the relationship between altered M1 activity and pain and disability, and 3) examine the M1 response to an acute exacerbation of muscle pain in an already sensitized system. It was hypothesized that progressively developing muscle soreness would result in M1 reorganization, reduced intracortical inhibition, and reduced IHI from the “affected” to the “unaffected” hemisphere, and these changes would be dependent on the magnitude and duration of muscle soreness. Consistent with previous studies (Le Pera et al. 2001; Schabrun and Hodges 2012; Schabrun et al. 2013), it was further hypothesized that an acute exacerbation of muscle pain in a sensitized system would reduce corticomotor output and increase intracortical inhibition.

Materials and Methods

Participants

Twelve, healthy, right-handed individuals (8 female, 26 ± 4 years, mean ± standard deviation [SD]) participated. Participants had no history of neurological or upper-limb conditions and completed a transcranial magnetic stimulation (TMS) safety screen (Keel et al. 2001) prior to commencement. All subjects received written and verbal description of experimental procedures and provided written informed consent consistent with the Declaration of Helsinki. Experimental procedures were approved by the local ethics committee (N-20130055).

Experimental Protocol

Each participant attended the laboratory on 4 occasions—Days 0, 2, 4, and 14 (Fig. 1). NGF was injected into the belly of the right extensor carpi radialis brevis (ECRB) muscle immediately following administration of quantitative sensory and motor testing (pressure pain thresholds [PPTs], muscle soreness, grip force, patient-rated tennis elbow evaluation [PRTEE], and short-form McGill Pain Questionnaire), and neurophysiological testing (motor cortical maps, corticomotor excitability, intracortical inhibition and facilitation, and IHI) on Days 0 and 2. To assess the immediate effect of the NGF injection on corticomotor output, corticomotor excitability was measured directly following the injection on Days 0 and 2. All outcome measures were repeated on Day 14, but no injection given.

To examine the effect of an acute exacerbation of muscle pain in the m. ECRB already sensitized by repeated NGF injection, an identical procedure to that described for Days 0 and 2 was followed on Day 4, except that the NGF injection was replaced with an injection of hypertonic saline to induce immediate experimental muscle pain. Measures of corticomotor excitability (motor-evoked potentials [MEPs]) and intracortical inhibition and facilitation were made during saline-induced muscle pain that lasted ∼10 min. Corticomotor excitability was reassessed once pain had returned to baseline levels. These data were compared with measures of corticomotor excitability and intracortical networks made in the NGF sensitized system immediately prior to saline-induced pain (i.e., data from the main protocol at Day 4).

Figure 1. Clinical (McGill Pain Questionnaire, PRTEE, muscle soreness, pressure algometry, and grip force) and neurophysiological (resting and active motor threshold, motor cortical maps, intracortical inhibition and facilitation, and IHI) outcome measures were assessed at the beginning of each experimental session on Days 0, 2, 4, and 14. On Days 0 and 2, these measures were followed by injection of NGF to right extensor carpi radialis brevis (m. ECRB) and corticomotor excitability immediately evaluated. On Day 4, hypertonic saline was injected to m. ECRB to induce acute pain lasting approximately 10 min. During acute pain, corticomotor excitability in the form of 15 MEPs and intracortical inhibition and facilitation were assessed. Once pain had returned to baseline, corticomotor excitability (15 MEPs) was re-evaluated.
NGF-Induced Muscle Soreness

Sterile solutions of recombinant human NGF were prepared by the pharmacy at Aalborg hospital. After cleaning the skin with alcohol, a dose of 5 μg (0.2 mL) was given as a bolus injection into the muscle belly of m. ECRB at Days 0 and 2 using a 1-mL syringe with a disposable needle (27G). The site of injection of m. ECRB was determined using real-time ultrasound guidance.

The PRTEE was used to assess average pain and disability of the injected arm at the start of each testing session (Days 0, 2, 4, and 14) (Macdonald 2005). Scores for pain (sum of 5 items out of a maximum score of 50) and disability (sum of 10 items, divided by 2, out of a maximum score of 50) were combined to give a total score ranging from 0 (no pain and no functional impairment) to 100 (worst pain imaginable with significant functional impairment).

Muscle soreness was assessed at the start of testing on Days 2, 4, and 14 using a modified 7-point Likert scale; 0 = “a complete absence of soreness,” 1 = “a light soreness in the muscle felt only when touched/vague ache,” 2 = “a moderate soreness felt only when touched/a slight persistent ache,” 3 = “a light muscle soreness when lifting or carrying objects,” 4 = “a light muscle soreness, stiffness or weakness when moving the wrist without gripping an object,” 5 = “a moderate muscle soreness, stiffness or weakness when moving the wrist,” and 6 = “a severe muscle soreness, stiffness or weakness that limits the ability to move” (Hayashi et al. 2013).

The short-form McGill Pain Questionnaire (Melzack 1987) was administered at the start of testing on Days 2, 4, and 14 to investigate the characteristics and distribution of NGF-induced pain. Subjects scored the 15 pain descriptors on the McGill Pain Questionnaire as “None,” “Mild,” “Moderate,” or “Severe.” Words chosen by at least one-third of participants were used in data analyses.

Saline-Induced Muscle Pain

Saline-induced pain was induced on Day 4 by a bolus injection of 0.5 mL of hypertonic saline (5.8%) into the muscle belly of m. ECRB after the skin had been cleaned with alcohol. Injections were performed using a 1-mL syringe with a disposable needle (27G). The site of injection of m. ECRB was determined using real-time ultrasound guidance. Pain was recorded every 30 s using an 11-point numerical rating scale (NRS: anchored with “no pain” at zero and “worst pain imaginable” at 10) immediately following hypertonic saline injection until pain returned to baseline. To capture the characteristics and distribution of pain induced specifically by hypertonic saline, the McGill Pain Questionnaire was administered once pain had returned to baseline.

Pressure Algometry

Pressure was applied at a rate of 30 kPa/s perpendicular to the surface of the skin using a handheld algometer (1-cm² probe, Somedic) covered by a disposable latex sheath. Three readings at the PPT were made at 1-min intervals, at 4 sites: 1) right m. ECRB (injection site), 2) left m. ECRB, 3) right m. Tibialis Anterior, and 4) left m. Tibialis Anterior. For each site, the muscle belly was located and marked. The PPT was defined as the point at which a sensation of pressure changed to a sensation of pain. Participants were requested to push a button when the pressure sensation first became painful. The average PPT of the 3 measures was used for statistical analysis. A tape measure was used to measure the position of each site (m. ECRB—distance (cm) distal to the lateral epicondyle and medial distance (cm); m. Tibialis Anterior—distance (cm) distal from the base of the patellar tendon), and these values were recorded to ensure consistent positioning across measurement sessions.

Grip Force

Maximal voluntary grip force was recorded using an electronic digital dynamometer (MIE Medical Research Ltd.). Participants positioned their forearm in pronation and 90 degrees elbow flexion. Peak values determined maximal grip force and were found following a single maximal voluntary effort lasting 10 s on each side. The force signal was sampled at 500 Hz.

Corticomotor Excitability

Electromyographic (EMG) activity was recorded from right and left m. ECRB using silver/silver chloride surface electrodes (Medicotest 720-01-K, Ambu A/S) positioned over the muscle belly. EMG signals were sampled at 4 kHz and bandpass filtered at 10 Hz–2 kHz. Data were digitized by a 16-bit data-acquisition card (National Instruments, NI6122) and saved by custom-made Labview software (Mr. Kick, Knud Larsen, SMI, Aalborg University).

Single-pulse transcranial magnetic stimuli (TMS) were delivered using a Magstim 200 stimulator (Magstim Co. Ltd) and a figure-of-eight coil. The coil was positioned over the left hemisphere at a 45-degree angle to the sagittal plane to preferentially induce current in a posterior-to-anterior direction. The optimal cortical site ("hotspot") to evoke responses in right m. ECRB was determined as the coil position that evoked a maximal peak-to-peak MEP for a given stimulation intensity. All TMS procedures adhered to the TMS checklist for methodological quality (Chippchase et al. 2012). With the participant seated, 3 measures were made:

1. Resting motor threshold (rMT), defined as the minimum stimulator intensity at which 5 out of 10 stimuli applied at the optimal scalp site evoked a response with a peak-to-peak amplitude of at least 50 μV.
2. Active motor threshold (aMT), defined as the minimum stimulator intensity at which 5 out of 10 stimuli evoked a response amplitude of 200 μV while m. ECRB was contracted at 10% of maximum voluntary contraction force. The arm was positioned with the elbow in 90 degrees flexion and the wrist pronated. Force feedback was provided via an oscilloscope positioned in front of the participant, with the target force of 10% marked. Participants were asked to maintain their force on the marked line at all times.
3. Fifteen MEPs were recorded at 120% of rMT over the optimal cortical site with m. ECRB at rest to evaluate corticomotor excitability. MEP responses were measured as peak-to-peak amplitudes and averaged for analysis.

Motor Cortical Maps

The procedure for cortical mapping has been described in detail previously (Schabrun and Ridding 2007; Schabrun et al. 2009). Participants were fitted with a cap, marked with a 1 x 1 cm grid and orientated to the vertex (point 0,0). The stimulus intensity for mapping was 120% rMT. TMS was applied every 6 s with a total of 5 stimuli at each site. The number of scalp sites was pseudorandomly increased until no MEP was recorded (defined as <50-μV peak-to-peak amplitude in all 5 trials in all border sites (Schabrun and Ridding 2007; Schabrun et al. 2009).
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Participants were seated and instructed to maintain their hand and forearm relaxed with the wrist pronated throughout the experiment. Trials containing background EMG activity were discarded.

The number of active map sites and map volume was calculated. A site was considered “active” if the mean peak-to-peak amplitude of the 5 MEPs evoked at that site was greater than 50 µV. The mean peak-to-peak MEP amplitudes at all active sites were summed to calculate the map volume. The center of gravity (CoG) was defined as the amplitude-weighted center of the map (Wassermann et al. 1992; Uy et al. 2002) and was calculated for each muscle using the formula:

$$\text{CoG} = \frac{\sum V_i \cdot X_i}{\sum V_i} = \frac{\sum V_i \cdot Y_i}{\sum V_i}$$

where $V_i$ represents mean MEP amplitude at each site with the coordinates $X_i$, $Y_i$. The reliability of these procedures for calculating the number of active sites, volume and center of gravity, and the stability of map measures over time has been previously demonstrated (Uy et al. 2002; Malcolm et al. 2006; Ngomo et al. 2012).

Finally, the number of discrete map peaks, defined as the number of scalp sites over which TMS evoked a discrete peak in the motor cortex representation, was determined. Motor cortical maps were normalized to the maximum MEP amplitude at Day 0 for each participant. Using an established procedure (Schabrun, Hodges et al. 2014; Schabrun, Jones et al. 2014), discrete peaks were identified if the MEP amplitude at a grid site was greater than 50% of maximum, was separated by a reduction in amplitude of at least 5% of peak MEP amplitude in 7 out of 8 of the surrounding grid sites, and was separated by at least 1 grid site from another peak that satisfied the first 2 criteria.

### Intracortical Inhibition and Facilitation

Short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were measured for right m. ECRB using a standard paired-pulse TMS protocol (Kujirai et al. 1993). Twelve trials were recorded at each of 2 interstimulus intervals—2 ms for SICI and 13 ms for ICF. The test stimulus was set to produce a MEP of between 0.3 and 0.4 mV peak-to-peak amplitude in relaxed m. ECRB, and the conditioning stimulus was set to 90% aMT (Ortu et al. 2008; Perez and Cohen 2008). To investigate short- and long-latency interhemispheric inhibition (SIHI and LIHI), 10- and 40-ms interstimulus intervals were selected (Nelson et al. 2009; Sattler et al. 2012). In pseudorandom order, 10 trials were recorded at each interstimulus interval and a further 10 trials using the test stimulus alone (30 trials in total). MEP responses were measured as peak-to-peak amplitudes and conditioned responses (SIHI/LIHI) expressed as a proportion of the unconditioned test response.

### Statistical Analyses

Muscle soreness scores, PRTEE, McGill Pain Questionnaire, and neurophysiological data (rMT, aMT, MEP amplitude at the hot-spot, active sites, map volume, discrete map peaks, CoG, SICI, ICF and LIHI) were compared between Days (0, 2, 4, and 14) using one-way repeated-measures analysis of variance (ANOVA). PPTs and maximal grip force were compared between Days (0, 2, 4, and 14) and sides (right, left) using two-way repeated-measures ANOVA. Data that did not meet assumptions of normality were log-transformed. Linear regression lines were used to assess the relationship between neurophysiological mechanisms of aMT, SICI/ICF, and IHI and quantitative variables of pain, disability (PRTEE), grip force, and pressure pain sensitivity.

The immediate effect of the NGF injection on corticomotor excitability output was examined using two-way repeated-measures ANOVA that compared corticomotor excitability data before and after NGF injection (factor “time”) on Days 0 and 2 (factor “day”). To assess the effect of saline-induced muscle pain on corticomotor excitability and intracortical networks (SICI/ICF), these measures were compared before, during, and after hypertonic saline infusion on Day 4 using one-way repeated-measures ANOVA.

Where appropriate, post hoc analyses were performed using Holm–Sidak multiple comparison tests. Statistical significance was set at $P < 0.05$. All data in text are presented as mean ± SD.

### Results

#### Sensory and Functional Measures in Response to Repeated NGF Injections

#### Pain and Disability

NGF-induced muscle soreness was local to the injection site in the majority of participants at Days 2 and 4 (Fig. 2). Two participants reported an ache that radiated to the upper arm and 2 to the lower forearm (radiating symptoms showed a similar distribution for these participants at Days 2 and 4 and were not present at Day 14). On the McGill Pain Questionnaire, NGF-induced muscle soreness was commonly described as a mild-to-moderate ache (33% of participants), heavy (33%), and/or tender (33%) at Day 2 and a mild-to-severe ache (58%), heavy (42%), tender (33%), stabbing (42%), and/or tiring (58%) at Day 4.

NRS scores corresponding to NGF-induced muscle pain (ANOVA: $F_{3,33} = 30.3; P < 0.001$) increased 2 days after the first injection compared with Day 0 ($P < 0.001$), increased further after the second injection from Day 2 to Day 4 ($P < 0.02$), and returned toward baseline scores at Day 14 (Fig. 3A).

PRTEE scores followed a profile similar to that of pain (ANOVA: $F_{3,33} = 23.9; P < 0.001$). PRTEE scores were increased at Day 2 compared with Day 0 ($P < 0.001$), elevated further at Day 4 compared with Day 2 ($P < 0.014$), and returned to baseline at Day 14 (Fig. 3B).

Likert scores of muscle soreness (ANOVA: $F_{3,33} = 27.9; P < 0.001$) were increased at Day 2 compared with Day 0 ($P < 0.001$) and
remained elevated at Day 4 compared with Day 0 ($P < 0.001$) with a trend toward an increase from Day 2 scores ($P < 0.065$). Muscle soreness returned to baseline at Day 14 (Fig. 3C). Grip Force

Grip force in the right (injected) arm was reduced at Day 4 (ANOVA interaction: $F_{3,33} = 3.20; P < 0.04$) compared with Day 0.
A greater reduction in grip force in the right arm was associated with a greater increase in active motor threshold at all time-points ($R = 0.42$, $P < 0.014$, Fig. 7A). Grip force was lower in the left arm when compared with the right arm at Day 0 ($P < 0.012$) and Day 2 ($P < 0.002$), but there was no difference between the two sides at Day 4 or Day 14. Grip force in the left arm was unaltered over time.

**Pressure Pain Sensitivity**
PPTs measured over the right (injected) m. ECRB were reduced (ANOVA interaction: $F_{3,33} = 20.6$; $P < 0.001$) at Day 2 ($P < 0.001$) and Day 4 ($P < 0.001$) compared with Day 0. There was a trend for PPTs to remain reduced at Day 14 ($P = 0.076$; Fig. 4). Interestingly, PPTs over the left m. ECRB were also reduced, although to a lesser extent than those for right m. ECRB ($P < 0.001$), at Day 4 compared with Day 0 ($P < 0.025$), returning to baseline at Day 14. PPTs over m. Tibialis Anterior were unaltered over time.

**Neurophysiological Measures in Response to Repeated NGF Injection**

$P$-values for significant post hoc tests are shown in the text below. $P$-values, $F$-values, and degrees of freedom (DF) from the primary ANOVA performed for each neurophysiological measure are provided in Tables 1–3.

### Motor Threshold and Motor Cortical Maps
Active, but not resting, motor threshold was increased at Day 4 compared with Day 0 ($P < 0.021$) but returned to baseline at Day 14 (Table 1).

Motor cortical maps at each time-point are provided in Figure 5. Compared with Day 0, map volume was increased at Day 4 ($P < 0.005$), and this was accompanied by an increase in the number of discrete map peaks at Day 4 ($P < 0.038$, Table 1). Neither parameter differed when compared with baseline at Day 14. There was no alteration in the amplitude of the MEP at the hotspot, the number of active sites, or the position of the CoG.

### Intracortical Networks
Consistent with the direction (increased excitability) and timing of changes in motor threshold and motor cortical maps, SICI was reduced, and ICF increased, at Day 4 (SICI, $P < 0.033$; ICF, $P < 0.004$; Table 1). These changes were not maintained at Day 14. The amplitude of the test response remained stable over time. Representative traces from 1 individual are provided in Figure 6. Greater

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**Figure 4.** Group data (mean ± SD, $N = 12$) for PPTs at m. ECRB and m. Tibialis Anterior at each time-point (Days 0, 2, 4, and 14). Each asterisk denotes a significant ($P < 0.05$) difference from baseline (Day 0). PPTs were reduced at Days 2 and 4 in the right (injected) m. ECRB and at Day 4 in left m. ECRB. No changes were observed in m. Tibialis Anterior at any time-point.

**Table 1.** Neurophysiological measures (mean ± SD, $N = 12$)

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 14</th>
<th>$P$-value</th>
<th>$F$-value</th>
<th>DF effect:error</th>
</tr>
</thead>
<tbody>
<tr>
<td>rMT (%)</td>
<td>43.2 ± 8.0</td>
<td>43.7 ± 7.7</td>
<td>45.7 ± 8.1</td>
<td>44.1 ± 8.1</td>
<td>0.19</td>
<td>1.67</td>
<td>3:33</td>
</tr>
<tr>
<td>aMT (%)</td>
<td>32.8 ± 8.4</td>
<td>34.3 ± 8.6</td>
<td>36.0 ± 8.8*</td>
<td>33.6 ± 8.5</td>
<td>0.024</td>
<td>3.60</td>
<td>3:33</td>
</tr>
<tr>
<td>MEP amplitude (mV)</td>
<td>0.28 ± 0.17</td>
<td>0.18 ± 0.12</td>
<td>0.21 ± 0.09</td>
<td>0.28 ± 0.33</td>
<td>0.29</td>
<td>1.31</td>
<td>3:33</td>
</tr>
<tr>
<td>Map active sites (number)</td>
<td>23.4 ± 8.9</td>
<td>19.8 ± 8.8</td>
<td>21.0 ± 8.9</td>
<td>23.5 ± 11.9</td>
<td>0.29</td>
<td>1.31</td>
<td>3:33</td>
</tr>
<tr>
<td>Map volume (mV)</td>
<td>2.6 ± 0.89</td>
<td>3.1 ± 2.0</td>
<td>4.6 ± 2.1*</td>
<td>3.3 ± 2.4</td>
<td>0.005</td>
<td>5.10</td>
<td>3:33</td>
</tr>
<tr>
<td>Map discrete peaks (number)</td>
<td>4.2 ± 1.6</td>
<td>4.1 ± 1.6</td>
<td>6.2 ± 2.6*</td>
<td>3.8 ± 1.5</td>
<td>0.009</td>
<td>4.57</td>
<td>3:33</td>
</tr>
<tr>
<td>CoG latitude (cm)</td>
<td>6.0 ± 0.69</td>
<td>5.5 ± 0.75</td>
<td>5.6 ± 0.80</td>
<td>5.6 ± 0.92</td>
<td>0.16</td>
<td>1.87</td>
<td>3:33</td>
</tr>
<tr>
<td>CoG longitude (cm)</td>
<td>4.6 ± 1.1</td>
<td>4.6 ± 0.97</td>
<td>4.9 ± 1.4</td>
<td>4.7 ± 1.30</td>
<td>0.71</td>
<td>0.46</td>
<td>3:33</td>
</tr>
<tr>
<td>SICI/ICF test response (mV)</td>
<td>0.31 ± 0.20</td>
<td>0.37 ± 0.22</td>
<td>0.38 ± 0.18</td>
<td>0.33 ± 0.20</td>
<td>0.62</td>
<td>0.60</td>
<td>3:33</td>
</tr>
<tr>
<td>SICI (proportion of test)</td>
<td>0.59 ± 0.25</td>
<td>0.64 ± 0.28</td>
<td>0.89 ± 0.29*</td>
<td>0.82 ± 0.25</td>
<td>0.012</td>
<td>4.37</td>
<td>3:33</td>
</tr>
<tr>
<td>ICF (proportion of test)</td>
<td>1.18 ± 0.32</td>
<td>1.14 ± 0.23</td>
<td>1.44 ± 0.40*</td>
<td>1.24 ± 0.34</td>
<td>0.001</td>
<td>6.81</td>
<td>3:33</td>
</tr>
</tbody>
</table>

*Note: P-values, F-values, and DF are from the primary ANOVA for each variable; $P < 0.05$ post hoc tests relative to baseline; rMT, resting motor threshold; aMT, active motor threshold; CoG, center of gravity in maps; SICI, short-interval intracortical inhibition; ICF, intracortical facilitation.*
Table 2 IHI measures (mean ± SD, N = 12)

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 14</th>
<th>P-value</th>
<th>F-value</th>
<th>DF effect : error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left hemisphere (right ECRB)</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Test response (mV)</td>
<td>0.37 ± 0.15</td>
<td>0.45 ± 0.29</td>
<td>0.46 ± 0.23</td>
<td>0.42 ± 0.20</td>
<td>0.98</td>
<td>0.06</td>
<td>3:33</td>
</tr>
<tr>
<td>IHI 10 ms (proportion of test)</td>
<td>0.59 ± 0.63</td>
<td>0.57 ± 0.36</td>
<td>0.51 ± 0.29</td>
<td>0.62 ± 0.35</td>
<td>0.90</td>
<td>0.19</td>
<td>3:33</td>
</tr>
<tr>
<td>IHI 40 ms (proportion of test)</td>
<td>0.63 ± 0.51</td>
<td>0.68 ± 0.46</td>
<td>0.57 ± 0.29</td>
<td>0.71 ± 0.37</td>
<td>0.76</td>
<td>0.39</td>
<td>3:33</td>
</tr>
<tr>
<td>Right hemisphere (left ECRB)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Test response (mV)</td>
<td>0.27 ± 0.14</td>
<td>0.33 ± 0.18</td>
<td>0.31 ± 0.15</td>
<td>0.32 ± 0.16</td>
<td>0.94</td>
<td>0.13</td>
<td>3:33</td>
</tr>
<tr>
<td>IHI 10 ms (proportion of test)</td>
<td>0.69 ± 0.36</td>
<td>1.30 ± 0.56</td>
<td>1.60 ± 0.92*</td>
<td>1.30 ± 0.54</td>
<td>0.022</td>
<td>3.78</td>
<td>3:33</td>
</tr>
<tr>
<td>IHI 40 ms (proportion of test)</td>
<td>0.73 ± 0.41</td>
<td>1.3 ± 0.46</td>
<td>1.4 ± 0.64*</td>
<td>1.2 ± 0.44</td>
<td>0.029</td>
<td>3.47</td>
<td>3:33</td>
</tr>
</tbody>
</table>

Note: P-values, F-values, and DF are from the primary ANOVA for each variable; *P < 0.05 post hoc tests relative to baseline; IHI, interhemispheric inhibition.

Table 3 Influence of hypertonic saline-induced pain at Day 4 on MEP amplitude and SICI/ICF (mean ± SD, N = 12)

<table>
<thead>
<tr>
<th></th>
<th>Before pain</th>
<th>During pain</th>
<th>After pain</th>
<th>P-value</th>
<th>F-value</th>
<th>DF effect : error</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEP amplitude (mV)</td>
<td>0.21 ± 0.09</td>
<td>0.34 ± 0.18*</td>
<td>0.37 ± 0.19*</td>
<td>0.015</td>
<td>5.10</td>
<td>2:22</td>
</tr>
<tr>
<td>Test response (mV)</td>
<td>0.38 ± 0.18</td>
<td>0.36 ± 0.27</td>
<td>—</td>
<td>0.17</td>
<td>2.17</td>
<td>1:11</td>
</tr>
<tr>
<td>SICI (proportion of test)</td>
<td>0.89 ± 0.29</td>
<td>0.80 ± 0.19</td>
<td>—</td>
<td>0.31</td>
<td>1.14</td>
<td>1:11</td>
</tr>
<tr>
<td>ICF (proportion of test)</td>
<td>1.44 ± 0.40</td>
<td>1.20 ± 0.27*</td>
<td>—</td>
<td>0.019</td>
<td>7.49</td>
<td>1:11</td>
</tr>
</tbody>
</table>

Note: P-values, F-values, and DF are from the primary ANOVA for each variable; *P < 0.05 post hoc tests relative to baseline; MEP, motor-evoked potential; SICI, short-interval intracortical inhibition; ICF, intracortical facilitation.

Figure 5. Averaged (N = 12) motor cortex maps normalized to the maximum MEP for each participant. Maps for Days 2, 4, and 14 are normalized to Day 0 for each participant. The colored scale represents the proportion of the maximum MEP amplitude of Day 0. The vertex is located at coordinate (0, 0). Note the increased map excitability (map volume) and larger number of discrete peaks following repeated NGF injection at Day 4.
ICF was associated with higher pain and disability on the PRTEE ($R = 0.37$, $P < 0.018$; Fig. 7B).

**Interhemispheric Networks**

The amount of IHI from the left (“affected”) to the right (“unaffected”) hemisphere (left, uninjected m. ECRB) was reduced at both short and long latencies at Day 4 (SIHI, $P < 0.016$; LIHI $P < 0.026$, Fig. 6). Lower IHI from the affected to the unaffected hemisphere at short- ($R = 0.30$, $P < 0.049$; Fig. 7C) and long-latency ($R = 0.91$, $P < 0.001$; Fig. 7D) was associated with reduced PPTs in left m. ECRB across all time-points. The amount of IHI from the unaffected to the affected hemisphere (right, injected m. ECRB) was unaltered across time at either latency. The amplitude of the test response in each muscle was stable over time (Table 2).

**The Immediate Effect of NGF Injection on Corticomotor Excitability**

Corticomotor excitability (ANOVA: $P < 0.044$) was unaltered immediately following the NGF injection on Day 0 (pre $0.28 \pm 0.17$ mV to $0.24 \pm 0.15$ mV post). However, once the system was sensitized on Day 2, the NGF injection produced an immediate increase (pre-NGF injection $0.18 \pm 0.12$ mV to $0.28 \pm 0.21$ mV post-NGF injection) in corticomotor excitability ($P < 0.034$).

**The Influence of an Acute Exacerbation of Muscle Pain on Corticomotor Excitability and Intracortical Networks in a NGF Sensitized System**

Injection of hypertonic saline induced an average muscle pain intensity of $7.6 \pm 1.8$ on the NRS and an average pain duration of $7.5 \pm 2.6$ min (range 5.0–12.5 min). The most frequent words used to describe the pain on the McGill Pain Questionnaire were stabbing (42%), sharp (58%), aching (42%), and heavy (50%). The majority of participants reported symptoms localized to the region of the injection. Three participants reported symptoms that radiated to the upper arm and 2 to the lower forearm. The injection of hypertonic saline at Day 4 into m. ECRB already sensitized by repeated NGF injection produced an increase in MEP amplitude during acute pain ($P < 0.049$), and this increase persisted after acute pain had returned to baseline ($P < 0.019$; Table 3). A reduction in ICF was present during acute pain (ANOVA: $P < 0.019$). There was no change in SICI or in the amplitude of the test response during pain.

**Discussion**

This study is the first to examine M1 in the transition to sustained muscle soreness. A novel feature is the use of a clinically realistic model that incorporates development of progressive muscle soreness with an acute exacerbation of muscle pain. The data
demonstrate altered M1 organization and impaired function characterized by increased corticomotor excitability (increased map volume, increased map peaks, reduced intracortical inhibition, and increased intracortical facilitation), that is present at Day 4 following progressively developing muscle soreness and is further potentiated by acute muscle pain. A unique, and previously unreported finding, was a reduction in IHI from the left ("affected") to the right ("unaffected") hemisphere, also present at Day 4, associated with greater pressure pain sensitivity in the unaffected arm. These findings provide original insight into the nature and temporal profile of M1 adaptation during the transition to sustained muscle pain.

**Temporal Profile of NGF-Induced Muscle Soreness and Disability**

A single injection of NGF at Day 0 induced muscle soreness, hyperalgesia to pressure, and reduced function of m. ECRB by Day 2. Repeated NGF injection resulted in a progressive increase in pain and disability, along with a reduction in grip force, at Day 4. All measures of pain, hyperalgesia, and disability, with the exception of grip force, returned toward baseline at Day 14. This temporal profile is usual following single (Bergin et al. 2014) and repeated NGF injection (Hayashi et al. 2013) with some studies reporting maintenance of symptoms up to 21 days (Dyck et al. 1997). These data confirm the suitability of repeated NGF injection as an experimental model to investigate the transition to sustained elbow pain.

A vehicle control was not included in the current study for several reasons. First, numerous studies demonstrate that measures of M1 organization and function are stable and reliable over time (Uy et al. 2002; Malcolm et al. 2006; Ngomo et al. 2012). Second, the use of such a control is common in both animal and human studies of experimental pain and rarely, if ever, shows an effect (Adachi et al. 2008; Nash et al. 2010). For instance, previous studies that have examined SICI/ICF in response to pain demonstrated no change in MEP amplitudes, SICI or ICF following a vehicle control (Fierro et al. 2010). Finally, in the present study, differential effects on M1 were observed over time (effects were not present at Day 2 and returned to baseline at Day 14). Taken together, these observations indicate that standard measures of M1 organization and function are not sensitive to time effects, and our findings are unlikely to be replicated in a no pain control condition.

**The Influence of Progressively Developing Muscle Soreness on M1 Organization**

Pain and disability evoked by repeated NGF injection-induced reorganization of M1 that was evident at Day 4. This temporal profile implies progressively developing muscle soreness is an...
important driver of M1 reorganization. However, although pain and disability were greater at Day 4 than Day 2, no association between M1 organization and pain severity/disability was found, suggesting sustained muscle soreness may be more important in driving reorganization than pain severity. Altered M1 organization was characterized by increased map volume and an increased number of discrete peaks in M1 activity. These findings provide evidence of an increase in corticomotor excitability that may reflect the search for a new motor strategy during the transition to sustained muscle pain.

The organizational structure of human M1 relies on a balance between discrete and distributed (overlapping) muscle representations (Devanne et al. 2006; Strother et al. 2012; Cunningham et al. 2013). This structure supports integrated, multi-joint, and synergistic movements while maintaining fine, individuated motor control (Dechent and Frahm 2003; Plassmann et al. 2010). Previous studies have shown that this structure is based on variation in the threshold of excitability such that each muscle has a lower threshold for excitation of key movements (observed as discrete peaks of excitability) whereas adjoining muscles not required for movement completion have a higher threshold (Humphrey and Freund 1991; Plassmann et al. 2010). The presence of multiple discrete peaks in the cortical representation of wrist muscles in the present study can therefore be interpreted to reflect intermuscle coordination required for different functions (Humphrey and Freund 1991; Scott and Kalaska 1997; Passingham et al. 2002), such as coordination between wrist extensor and finger flexor muscles. Consistent with recent theories on pain adaptation (Hodges and Tucker 2011), an increase in the number of discrete peaks may reflect the search for a new motor strategy that utilizes synergies with surrounding muscles to redistribute muscle activity and reduce loading on a painful structure.

The finding of increased map peaks in the transition from acute to maintained pain is in contrast to a reduced number of peaks observed in chronic elbow pain (lateral epicondylalgia) in cross-sectional studies (Schabrun, Hodges et al. 2014). This discrepancy may be explained by the long duration of pain (>6 weeks) used to classify individuals with chronic lateral epicondylalgia. Increased excitability and reorganization of motor cortical maps is known to occur in the early stages of motor learning (Pascual-Leone et al. 1994, 1995). However, once a new motor strategy is acquired and becomes more automatic, motor representations contract (Pascual-Leone et al. 1994). A similar pattern may exist in the early versus late stages of sustained muscle soreness as a new motor strategy is first sought and then adopted. Indeed, studies have shown increased variability in trunk muscle activation in acute experimentally induced back pain (Hodges et al. 2013) but decreased variability in individuals with chronic back pain (Falla et al. 2014).

**The Influence of Progressively Developing Muscle Soreness on Intracortical Networks**

This study is the first to examine cortical organization and inhibitory networks in tandem in response to progressively developing muscle soreness. Sustained muscle soreness reduced intracortical inhibition and increased intracortical facilitation. The direction (increased corticomotor excitability) and time-course (changes evident at Day 4) was similar to that observed for cortical reorganization. As cortical representations are known to be maintained and adjusted by GABAergic intracortical inhibitory networks (Liepert et al. 1998), altered intracortical activity is a plausible mechanism to explain M1 reorganization in the current study.

Previous studies have reported a reduction in intracortical inhibition (GABA, mediated) and facilitation (glutamate mediated) in chronic pain conditions such as low back pain (Masse-Alarie et al. 2012), fibromyalgia (Mhalla et al. 2010), and complex regional pain syndrome (Schwenkreis et al. 2003, Eisenberg et al. 2005). Yet, intracortical inhibition is increased (facilitation remains reduced) in response to acute muscle pain (Schabrun and Hodges 2012). This finding, coupled with data from the present study, suggests a reversal of inhibitory activity that develops early in the transition to sustained muscle soreness (Day 4) and persists when pain becomes chronic. Although the effect of sustained elbow pain on SICI and ICF in the present study is complementary, several authors have suggested that inhibitory and facilitatory networks act independently (Ziemann et al. 1996; Liepert et al. 1998). This may explain why ICF, but not SICI, was associated with greater pain and disability in the current study.

In contrast to data for cortical organization and intracortical networks, active motor threshold was increased at Day 4. This finding could be explained by decreased spinal motoneuron excitability, reduced corticospinal excitability, or a combination of both, during active contraction. As spinal motoneuron excitability was not measured in the present study, it is not possible to determine the relative contribution of cortical versus spinal mechanisms, and this limitation should be addressed in future work. Regardless of the precise mechanism, increased active motor threshold could explain the reduction in grip force on the affected side. In support of this hypothesis, the increase in active motor threshold in the current study was associated with reduced grip force on the affected side. This finding is consistent with previous studies of chronic low back pain where higher active motor threshold has been shown to be correlated with poorer synchronization between transversus abdominis and internal oblique muscle onset (Masse-Alarie et al. 2012) and with higher disability (Strutton et al. 2005). One consideration is whether the increase in active motor threshold contaminated the measurement of SICI and ICF. Although this possibility cannot be ruled out, an increase in active motor threshold would be expected to produce an underestimation of the effects observed in the present study (i.e., increased active motor threshold would be expected to reduce excitability).

**The Influence of Progressively Developing Muscle Soreness on IHI**

Bilateral sensorimotor dysfunction is observed in chronic unilateral pain conditions. For instance, individuals with unilateral lateral epicondylalgia display flexed wrist postures, increased upper-limb reaction times, reduced speed of movement, and increased pressure and thermal pain thresholds in the unaffected limb (Pienimaki et al. 1997; Bisset et al. 2006; Heales et al. 2014), indicating a role for supraspinal mechanisms. This study is the first to examine IHI in the transition to sustained muscle pain.

IHI was reduced from the left (“affected”) to the right (“unaffected”) hemisphere at both short and long latencies at Day...
4. There was no change in IHI from the right (“unaffected”) to the left (“affected”) hemisphere. There is good evidence that IHI is mediated via transcallosal pathways and is of cortical origin (Meyer et al. 1995; Di Lazzaro et al. 1999; Reis et al. 2008). Consistent with data from chronic lateral epiduralalgia, a reduction in PPTs was found in the unaffected left m. ECRB at Day 4. As PPTs are reliable when recorded over multiple days (Nuesbaum and Downes 1998), this effect is unlikely to be due to the repeated-measures design.

A particularly novel finding was the association between a greater reduction in IHI at both short and long latency and the reduction in PPTs in left m. ECRB. These data provide the first evidence of a release of inhibition from the affected to the unaffected hemisphere during the transition to sustained muscle pain that could underpin bilateral changes in sensorimotor motor dysfunction observed in chronic pain states. Although the precise mechanism is unknown, it is possible that a release of IHI influenced PPTs on the unaffected side through reduced inhibition of thalamic neurons. It is well documented that M1 stimulation relieves pain, an effect likely mediated via corticothalamic projections that induce suppression of sensory information relayed in the spinothalamic tract (LeFauchoeur et al. 2006; Lucas et al. 2011). In addition, imaging studies reveal effects of M1 stimulation on other pain-processing regions including the anterior cingulate cortex, orbitofrontal cortex, insula, secondary sensory cortex, and periaqueductal gray matter (PAG) (Garcia-Larrea et al. 1999; Garcia-Larrea and Peyron 2007; Peyron et al. 2007). One possibility is that release of IHI in the unaffected hemisphere reduces downstream inhibition of thalamic neurons, the PAG, and/or other pain-processing regions, increasing sensitivity to pressure stimuli. However, the relationship between altered M1 excitability and pain sensitivity has not yet been characterized. This hypothesis requires further investigation before the specific mechanistic pathways can be disentangled.

The Effect of an Acute Exacerbation of Muscle Pain in a NGF Sensitized System

The induction of an acute exacerbation of muscle pain in a system already sensitized by NGF produced opposite effects on corticomotor excitability, but similar effects on intracortical networks, to those seen when acute muscle pain is induced in healthy individuals (Le Pera et al. 2001; Svensson et al. 2003; Martin et al. 2008; Schabrun and Hodges 2012). Increased corticomotor excitability was observed during and after acute muscle pain, and this effect was accompanied by a reduction in ICF, but no change in SICI, during pain. The reduction in ICF, and no change in SICI, during pain is consistent with previous studies of hypertonic saline injection in healthy individuals (Schabrun and Hodges 2012). Why corticomotor excitability was increased in the NGF sensitized system is unclear. However, as individually recorded MEPs provide a measure of excitability of the entire pathway from cortex to muscle, 1 possibility is that excitation observed in the NGF sensitized system reflects changes occurring at the level of the spinal motoneuron. These data indicate that an acute exacerbation of muscle pain in a sensitized system may increase spinal excitability without concomitant changes in motor cortical excitability. This finding may have relevance for clinical pain states that display acute exacerbation of muscle pain in the presence of ongoing muscle soreness. Future studies should seek to clarify the contribution of spinal motoneuron excitability to the findings outlined in this study.

Conclusions

Primary motor cortical organization and function are altered in the transition to sustained muscle soreness and are further potentiated by an acute exacerbation of muscle pain. Changes become evident after 4 days of progressing intensity of muscle soreness. Altered organization is characterized by increased map volume and an increased number of map peaks and is accompanied by altered function of intracortical networks (reduced intracortical inhibition and increased ICF). A release of IHI from the “affected” to the “unaffected” hemisphere occurs at Day 4 and is accompanied by a reduction in PPTs in the asymptomatic m. ECRB. These findings provide the first insight into the nature and temporal profile of M1 adaptation in the transition to sustained muscle pain. This information may have relevance for the development of therapeutic interventions that seek to M1 in the transition period.

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Notes

Conflict of Interest: None declared.

References


