Intracortical motor networks are affected in both the contralateral and ipsilateral hemisphere during single limb cold water immersion.

Author

Delahunty, Eden T, Bisset, Leanne M, Kavanagh, Justin J

Published

2019

Journal Title

Experimental Physiology

Version

Accepted Manuscript (AM)

DOI

10.1113/EP087745

Intracortical motor networks are affected in both the contralateral and ipsilateral hemisphere during single limb cold water immersion

Eden T Delahunty [1], Leanne M Bisset [1], and Justin J Kavanagh [1]

[1] Menzies Health Institute Queensland, Griffith University, Gold Coast campus, Queensland, Australia

Corresponding author: Eden Delahunty

School of Allied Health Sciences

Griffith University, Gold Coast
Queensland 4222, Australia

eden.delahunty@griffithuni.edu.au
Tel: +61 (7) 5552 8057
Fax: +61 (7) 5552 8674

NEW FINDINGS

What is the central question of this study?

How does single limb cold water immersion affect corticomotor function and intracortical circuitry in the motor cortex of each cerebral hemisphere?

What is the main finding and its importance?

Immersion of a single limb in very cold water caused an increase in corticomotor excitability and intracortical facilitation, and a decrease in intracortical inhibition, in the motor cortex of both hemispheres. These findings provide evidence that intense sensory stimuli induce widespread changes in motor circuitry in the contralateral, as well as the ipsilateral, hemisphere.

ABSTRACT

Although responses to noxious stimuli have been extensively studied for the contralateral hemisphere, little is known about how the ipsilateral hemisphere may be affected. Therefore, this study examined how exposing a single limb to noxious cold stimuli affects motor output arising from both the contralateral and ipsilateral hemisphere. A total of 17 healthy adults participated in three experiments. Single- and paired-pulse TMS protocols were used to identify how immersing a single upper limb in cold water (4.0 ± 0.5 ºC) affects inhibitory and facilitatory circuits in the primary motor cortex (M1) of the contralateral (experiment 1) and ipsilateral (experiment 2) hemisphere. The third experiment used a reaction time task to assess the functional consequences of acute adaptations in the ipsilateral M1. The target muscle in all experiments was the extensor carpi radialis brevis (ECRB). Immersion of a single limb in cold water increased self-perception of pain and temperature, and increased EMG amplitude of the immersed limb. During immersion, motor evoked potentials and intracortical facilitation increased, whereas short interval intracortical inhibition decreased, for both the ipsilateral M1 and contralateral M1. Activity in the ipsilateral hemisphere to the limb immersed in cold water also slowed reaction time for the non-immersed limb. Our findings suggest that altered motor responses from single limb cold water immersion are not restricted to a single hemisphere. Instead, widespread activation of somatosensory systems influences inhibitory and facilitatory circuits in the primary motor cortex of each hemisphere.

Keywords. Ipsilateral motor cortex; inhibition; facilitation; reaction time; cold water immersion.

1. INTRODUCTION

Although the mechanisms underlying musculoskeletal pain are complex, a common characteristic of exposure to pain is altered motor cortex neuromodulation. Sensory inputs to the motor cortex are diverse, so increasing activity in pain-associated pathways at the cortical, sub cortical, or spinal levels has the potential to also change activity in motor pathways (Ziemann, 2001). Indeed, investigations that induce moderate-to-severe muscle pain via hypertonic saline injection and capsaicin have consistently revealed that motor evoked potentials (MEP) from the contralateral primary motor cortex (M1) are suppressed with pain (Burns, Chipchase, & Schabrun, 2016; Fierro et al., 2010; Le Pera et al., 2001; Martin, Weerakkody, Gandevia, & Taylor, 2008; Mercier, Gagne, Reilly, & Bouyer, 2016; Schabrun & Hodges, 2012; Schabrun, Jones, Kloster, & Hodges, 2013; Svensson, Miles, McKay, & Ridding, 2003).

Under conditions where moderate or severe levels of perceived pain are induced, increased activity occurs in contralateral short interval intracortical inhibition (SICI) networks (Fierro et al., 2010; Schabrun & Hodges, 2012). Similarly, there is increased activity in contralateral SICI networks during time points following very brief (≤ 90 s), pain-inducing, immersion of a hand in cold water (Salo, Vaalto, Koponen, Nieminen, & Ilmoniemi, 2019). This feature is particularly evident following the resolution of pain and is proposed to occur via enhanced $GABA_A$ receptor activity (Di Lazzaro et al., 2000; Ziemann, Lonnecker, Steinhoff, & Paulus, 1996). In some situations, altered neuromodulation in the contralateral M1 is not restricted to inhibitory networks. Instead, MEP suppression appears to be a factor of enhanced SICI and reduced intracortical facilitation (ICF) (Fierro et al., 2010), where reductions in ICF are typically associated with decreased glutamatergic interneuron activity, and *N*-methyl-$_D$-aspartate (NMDA) receptor-mediated activity (Schwenkreis et al., 1999; Ziemann, Chen, Cohen, & Hallett, 1998). Although responses to induced localised pain have been studied for

the contralateral hemisphere, remarkably little is known about how noxious stimuli affects motor pathways arising in the ipsilateral hemisphere. This is surprising given that neuroimaging studies suggest that the locus for pain-related responses is not restricted to a single hemisphere.

Haemodynamic responses obtained from PET and fMRI indicate that regional blood flow is elevated in secondary somatic areas, insular regions, and thalamic regions of both hemispheres following the application of noxious stimuli to a single limb (Casey, Minoshima, Morrow, & Koeppe, 1996; Coghill, Sang, Maisog, & Iadarola, 1999; Peyron et al., 1999). More recent work indicates that cutaneous and muscle injection of noxious stimuli in a limb evokes bilateral activity in the ventral posterior thalamus as well as primary and secondary somatosensory cortices (Nash et al., 2010a, 2010b). The motor cortex is less frequently a focus of pain-related neuroimaging research, so there is less data available for the ipsilateral M1. Of the limited data that is available (Henderson, Bandler, Gandevia, & Macefield, 2006; Loggia et al., 2012; Macefield, Gandevia, & Henderson, 2007; Maeda et al., 2011; Nash et al., 2010a, 2010b; Niddam et al., 2002; Takahashi et al., 2011; Uematsu, Shibata, Miyauchi, & Mashimo, 2011) only a single MRI experiment reports increased blood oxygen level dependent (BOLD) activity in the ipsilateral M1 in response to single limb pain (Henderson et al., 2006). While this provides some evidence that widespread changes in M1 activity occurs with localised pain, it is unclear if this ipsilateral BOLD response reflects an increase or decrease in M1 output to target muscles.

The purpose of this study was to examine how increased sensory input to a single limb affects motor networks in both cerebral hemispheres. Three experiments were performed where a single limb was immersed in cold water in each study to elevate noxious sensation. In the first two experiments, measurements of cortical inhibition and facilitation were recorded from the contralateral M1 and ipsilateral M1. It was predicted that enhanced sensory

input (via single limb cold water immersion) to one side of the CNS would cause excitability changes in each motor cortex. An EMG-based reaction time experiment was also performed to explore functional consequences of changes to the ipsilateral M1. It was hypothesised that elevated noxious sensation would delay the time it takes to activate the target muscle from a resting state.

2. METHODS

2.1. Ethical Approval

Written, informed consent was obtained prior to performing any testing. This study acquired ethical approval from the Griffith University Human Research Ethics Committee (GU Ref No: 2017/507), and all testing procedures were conducted in accordance with the Declaration of Helsinki, with the exception of registration in a database.

2.2. Participants

A total of seventeen healthy subjects participated in three experiments (experiment 1: n = 8, 7 male, age = 21.8 ± 1.7 years; experiment 2: n = 8, all male, age = 22.1 ± 2.7 years; experiment 3: n = 10, 7 males, age = 21.1 ± 1.2 years). Two individuals participated in all experiments, and six participated in the second and third experiment. Participants were excluded if they used CNS active drugs, had a history of neurological dysfunction, or had any current/recent injury or pain syndromes. The dominant upper limb for each participant was determined using the Oldfield Edinburgh Inventory.

2.3. Experimental design

The three experiments were human, within-subject, cross-over designs (Figure 1). Experiment 1 examined whether immersion of a single limb in cold water influenced resting motor threshold (rMT), motor evoked potentials (MEP), inhibitory circuits (SICI, LICI), and facilitatory circuits (ICF) of the contralateral hemisphere. Experiment 2 examined whether immersion of a single limb in cold water affected the same TMS-based measurements for the ipsilateral hemisphere. Experiment 3 used an upper limb reaction time task to determine if the cold water immersion intervention influenced voluntary muscle activation in opposite limb. Extensor carpi radialis brevis (ECRB) was the test muscle in all experiments. The configuration of participants for each experiment is illustrated in Figure 1.

2.4. Experimental arrangement

Participants were seated comfortably in a chair with their head and neck supported. The dominant limb was rested on a custom designed apparatus which placed the wrist in neutral, the forearm in pronation, the elbow at 90° flexion, and the shoulder in 30° abduction. The non-dominant limb was placed in the same orientation but was rested in a water bath throughout testing. The bath was empty for the control condition and was filled with chilled water (4.0 ± 0.5 ºC) for the experimental condition. The water level was controlled so that only the hand and forearm was immersed. The total immersion time of the limb was less than 20 minutes for each experiment.

A cold water immersion acclimation period of five minutes was provided for each participant. Pilot testing revealed that this period allowed the participants to reach a steady state of perceived pain and cold temperature sensation, and thus the TMS and reaction time

measurements could be obtained under consistent conditions. Perceived pain and cold temperature intensity were rated on a modified 11-point numerical rating scale (NRS), with 0 indicating 'no pain' and 'not cold at all', and 10 indicating the 'worst pain imaginable' and 'coldest imaginable temperature sensation'. Pain and temperature NRS were recorded at 30-second intervals during the acclimation period, and immediately after completing the TMS or reaction time protocols. The order that the participants performed the control and cold water immersion conditions was counterbalanced, and a minimum of 2 hours recovery was provided to each participant who performed the cold water immersion condition first.

2.5. Electromyography

Surface EMG was recorded from ECRB using Ag-AgCl bipolar surface electrodes (24 mm diameter, Kendall Arbo, Germany). The first surface EMG electrode was placed approximately 2 cm distal to the lateral epicondyle, with the second surface electrode placed distally with an inter-electrode distance of 24 mm. For experiment 1 and 2, ECRB EMG data was sampled at 2000 Hz using a Power1401-mkII acquisition system and Signal software (CED, Cambridge, UK). EMG was amplified 10,000 times using a NL844 differential amplifier, and bandpass filtered at 10-500 Hz using NL135 and NL144 filters (Digitimer Ltd, UK). For experiment 3, EMG data was sampled at 1000 Hz, amplified 1,000 times, and bandpass filtered between 3 and 500 Hz using the same EMG system.

2.6. Cortical stimulation

Focal TMS was applied to the M1 contralateral to the limb undergoing cold water immersion for experiment 1, and the M1 ipsilateral to the limb undergoing cold water immersion for experiment 2. A figure-of-eight stimulating coil (internal wing diameter 70 mm) was used in

combination with two Magstim 200 magnetic stimulators connected with a BiStim unit (Magstim, Dyfed, UK). A posterior-to-anterior current flow was transynaptically induced by orienting the stimulating coil tangentially to the surface of the head, with the handle pointing in the posterior direction 45° to the inter- hemispheric line. The optimal coil location was identified as the M1 position that elicited the largest MEP in the test limb ECRB EMG following a slightly suprathreshold single-pulse TMS.

2.7. Single-pulse and paired-pulse protocol

Resting motor threshold (rMT) was determined as the minimum stimulation intensity required to obtain a single-pulse MEP of 50 microvolts (μV) for ECRB in at least 5 out of 10 trials. The rMT was measured for both the control and the single limb cold water immersion condition following acclimation. The suprathreshold test stimulus (TS) for all TMS-based measurements was set to 120% rMT. A series of unconditioned TS were delivered to obtain MEP data.

Standard paired-pulse TMS protocols were used which consisted of a subthreshold stimulus of 80% rMT being used as the conditioning stimulus (CS) for both SICI and ICF (Kujirai et al., 1993), and a suprathreshold CS of 120% rMT for LICI (Valls-Sole, Pascual-Leone, Wassermann, & Hallett, 1992). SICI was assessed by delivering the TS at an ISI of 2 ms following the subthreshold CS. The TS for ICF was delivered at an ISI of 10 ms following the subthreshold CS. The TS for LICI was delivered at an ISI of 100 ms following the suprathreshold CS. A minimum of 10 s occurred between successive measurements, and the order of MEP, SICI, ICF and LICI administration was counterbalanced to avoid order effects.

2.8. Reaction time protocol

The reaction time protocol in experiment 3 was a modified simple reaction time task, where participants were required to extend their wrist as fast as possible in response to a standardised auditory tone. The 100 ms tone was generated with custom Spike2 software and was presented at random intervals between 3 and 8 s. Participants were encouraged to relax between trials so that background EMG was minimised between reactive movements. Forty consecutive reaction time trials were performed for both the control and cold water immersion intervention conditions (i.e. a total of 80 trials for the experiment).

2.9. Data analysis

All analyses were performed in Signal 6.04 (CED, Cambridge, UK). MEP latency was the duration (ms) from TS to the first deflection of the MEP. The peak-to-peak amplitude (mV) of unconditioned and conditioned test MEPs was calculated for the control and cold water immersion conditions. Inhibition and facilitation were calculated as the ratio of the mean conditioned to the mean unconditioned MEP amplitudes for each subject. A MEP ratio less than one indicated inhibition, whereas a ratio greater than one indicated facilitation. Reaction time was the duration between the auditory tone and the onset of activity in full wave rectified ECRB EMG. Onset of activity was determined by visual inspection, which has been established as being as accurate and reliable as computer-based algorithms applied to the same reaction time methods (Hodges & Bui, 1996; Kavanagh, Bisset, & Tsao, 2012).

2.10. Statistical analysis

Pain and temperature intensity ratings were examined using one sample t-tests and one-way repeated measures ANOVA. Mixed model two-way ANOVA was used to assess rMT, where factors of intervention (control, cold water immersion) and hemisphere (contralateral, ipsilateral) were entered into the model. Tukeys post-hoc analyses were conducted if interaction effects were identified. As the resting ECRB EMG rms and the remaining TMS-derived variables were not normally distributed (assessed using Shapiro-Wilk test), a non-parametric Friedman's two-way analysis of variance by ranks test was used in place of the parametric two-way ANOVA. Post hoc analysis was conducted with the Wilcoxon signed ranks test, which were also applied to reaction time data to determine whether there were any differences in reaction time between the cold water immersion and control conditions. Significance was set at $p < 0.05$ for all comparisons.

3. RESULTS

3.1. Pain and temperature ratings

The profile of pain and temperature perception due to cold water immersion were the same between experiments, so 11-point NRS will only be reported for experiment 1. Both pain and cold temperature intensity ratings were significantly greater than the reference value of 0 (no pain) for all time intervals during the acclimation phase of the cold water immersion condition (all p's < 0.001, Figure 2). One-way repeated measures ANOVA revealed a main effect of time for pain intensity ($F(9, 63) = 5.369$, $p < 0.001$) and temperature intensity ($F(9, 63) = 5.615$, $p < 0.001$), where planned comparisons revealed a significant difference in pain intensity ($p = 0.002$) and temperature intensity ($p = 0.033$) between the 30 s and 60 s following cold water immersion.

One-way ANOVA revealed that the pain and cold temperature intensity ratings at the completion of the experiment were not significantly different to those recorded at the end of the acclimation period. Although formal interviews were not conducted, 82% of participants expressed extreme discomfort during testing, and 53% expressed that they would not have been able to continue with their limb immersed for even another 2 min.

3.2. Resting Motor Threshold and resting ECRB EMG amplitude

There were no significant differences between control and cold water immersion conditions for the intensity of the stimulator output ($p = 0.742$), and no differences between contralateral and ipsilateral hemispheres for rMT ($p = 0.151$, Table 1). Friedman's two-way analysis of variance by ranks test identified a significant difference for the resting ECRB EMG rms amplitude which depended on the intervention and the hemisphere that was examined (X^2 (3) = 35.710, $p < 0.001$). Wilcoxon signed ranks test revealed that there were significant differences for both the control ($Z = -3.331$, $p = 0.001$) and cold water immersion ($Z = -3.312$, $p = 0.001$) conditions when comparing the contralateral and ipsilateral hemisphere (Table 1). There was also a difference detected between the control and cold water immersion conditions for the contralateral hemisphere. No ECRB EMG differences were detected between the control and cold water immersion conditions for the ipsilateral hemisphere.

3.3. Single-pulse TMS trials

A significant difference was detected for MEP amplitude which depended on the intervention and hemisphere being examined (X^2 (3) = 13.950, $p = 0.003$). MEP amplitude for the cold water immersion condition was greater than the control condition for both the contralateral (*Z*

= –2.521, $p = 0.012$) and ipsilateral hemisphere ($Z = –2.100$, $p = 0.036$, Figure 3.A). No between-hemisphere differences were found for the control ($Z = –1.540$, $p = 0.123$) or the cold water immersion condition ($Z = –1.680$, $p = 0.093$). The latency of MEPs did not differ with the intervention or the hemisphere that was examined ($p = 0.416$, Figure 3.B).

3.4. Paired-pulse TMS trials

Significant differences were detected for SICI and ICF that depended on the intervention and hemisphere that was examined (SICI: X^2 (3) = 9.900, $p = 0.019$ ICF: X^2 (3) = 14.550, $p = 0.002$, LICI: X^2 (3) = 3.800, $p = 0.284$). For SICI measurements, there was less inhibition for the cold water immersion condition compared to the control condition for the contralateral hemisphere ($Z = –2.100$, $p = 0.036$) and the ipsilateral hemisphere ($Z = –2.380$, $p = 0.017$, Figure 4.A). In addition, between-hemispheres difference emerged for SICI during the control condition. When no intervention was present, the ipsilateral hemisphere exhibited increased inhibition compared to the contralateral hemisphere ($Z = –2.240$, $p = 0.025$). There were no significant between-hemisphere differences for SICI when the single limb was immersed in cold water ($Z = –1.820$, $p = 0.069$). ICF measurements indicated that there was greater facilitation for the cold water immersion condition compared to the control condition for the contralateral hemisphere ($Z = –2.100$, $p = 0.036$) and the ipsilateral hemisphere ($Z = –2.380$, $p = 0.017$, Figure 4.B). In contrast to SICI, a between-hemispheres difference emerged for ICF during the cold water immersion condition. While there was no significant difference between-hemispheres for the control condition ($Z = –1.260$, $p = 0.208$), facilitation was greater in the ipsilateral hemisphere when a single limb was immersed in cold water ($Z = –2.521$, $p = 0.012$).

3.5. Reaction time

Wilcoxon signed-ranks testing revealed that reaction time was significantly faster in the control (25^{th} percentile: 119 ms; 75^{th} percentile: 142 ms) condition compared to the cold water immersion (25^{th} percentile: 129 ms; 75^{th} percentile: 152 ms) condition ($Z = 2.60$, $p = 0.009$, $r = 0.82$, Figure 5).

4. DISCUSSION

We have found for the first time that extended immersion of a single limb in cold water 1) only increased EMG amplitude of the immersed limb, 2) increased MEP amplitude in both hemispheres, 3) reduced activity in inhibitory motor networks of both hemispheres, and 4) increased activity in facilitatory motor networks of both hemispheres. Given that cold water immersion of a single limb also compromised reaction time of the opposite limb, this study provides strong evidence that localised noxious sensation can cause widespread changes in motor activity in healthy individuals.

4.1. Resting muscle activity is only affected in the limb exposed to cold water immersion

Although resting ECRB EMG increased for the limb that was immersed in cold water, ECRB activity for the non-immersed limb remained the same during the intervention. As such, the observed increases in MEP amplitude for the non-immersed limb most likely reflect adaptation in cortical networks rather than factors such as muscle potentiation. With this in mind, it is not surprising that MEP amplitude for the contralateral hemisphere increased

during the cold water immersion condition compared to control due to the increased ECRB EMG activity at rest. In particular, for stimulator intensities set 20% above motor threshold, increases in background muscle activity via as little as 1.5% MVC can induce a 3-fold increase in compound muscle action potential amplitude compared to relaxed muscle (Hess, Mills, & Murray, 1987).

4.2. Single limb cold water immersion increased corticomotor excitability in the ipsilateral and contralateral M1

Studies that assess the effects of acute pain on corticomotor excitability have consistently observed MEP suppression for the contralateral hemisphere (Burns et al., 2016; Fierro et al., 2010; Le Pera et al., 2001; Martin et al., 2008; Mercier et al., 2016; Schabrun & Hodges, 2012; Schabrun et al., 2013; Svensson et al., 2003; Valeriani et al., 2001). Our opposing results may be due to our intervention, which elicited nociception using a different modality. The majority of acute interventions elicit pain via injection to localised areas such as small hand (Burns et al., 2016; Le Pera et al., 2001; Schabrun & Hodges, 2012; Schabrun et al., 2013; Svensson et al., 2003), forearm (Le Pera et al., 2001) and upper arm muscles (Martin et al., 2008), via laser stimulation to the dorsal hand (Valeriani et al., 2001), or via transient thermal heat stimulation (at 50 °C) to the side of the hand (Mercier et al., 2016). However, participants in the current study had a comparatively larger surface area exposed to the stimuli, with several participants reporting high levels of pain and cold temperature perception on the NRS scale. Immersing a limb in cold water of 4 degrees Celsius is known to cause a cutaneous response (Cain, Khasabov, & Simone, 2001; Darian-Smith, Johnson, & Dykes, 1973; Koltzenburg, Stucky, & Lewin, 1997; Schepers & Ringkamp, 2010), much like the pain-related effects seen when applying capsaicin to the skin (Ringkamp et al., 2001;

Schmelz, Schmid, Handwerker, & Torebjork, 2000). However, motor responses observed following cutaneous stimulation, and in particular capsaicin application, typically align with MEP suppression (Fierro et al., 2010). Instead, our MEP results are more consistent with deafferentation that may occur following ischemia or cuff occlusion.

By applying a cuff at the elbow (inflated to 220-250 mmHg) to induce a complete nerve block, MEP amplitude in muscles proximal to the tourniquet increase by approximately 30% from baseline (Ziemann, Corwell, & Cohen, 1998). Although this is a greater effect compared to the ~16% increases in MEP amplitude observed in the current study, it should be recognised that limb cooling merely reduces nerve conduction velocity in the immersed limb instead of causing complete nerve block (Herrera, Sandoval, Camargo, & Salvini, 2010). Given that subcortical and spinal excitability are not altered during cuffing (Brasil-Neto et al., 1993), it is likely that the increase in MEP amplitude reflects changes at the cortical level. It should also be noted that during brief acute pain interventions, such as the cold pressor test, muscle sympathetic nerve activity increases in combination with heart rate and blood pressure (Fagius, Karhuvaara, & Sundlof, 1989; Yamamoto, Iwase, & Mano, 1992). Therefore, it cannot be ruled out that our intervention also evoked changes in the autonomic nervous system which ultimately affected motor pathways.

4.3. Intracortical inhibitory and facilitatory networks

Immersion of a single limb in cold water decreased activity in SICI networks, but not LICI networks, for both the contralateral and ipsilateral hemispheres. Although SICI has been reported to increase with brief cold water exposure in a recent study (Salo et al., 2019), it is important to note differences in the time course of stimuli exposure across studies. The increased activity of SICI found by Salo et al. (2019) were during the post-cold conditions

following withdrawal of the hand from the cold water, whereas the changes to SICI for the current study were observed during the cold water immersion condition. Furthermore, the differential findings may be partly due to the length of immersion in cold water, which was ≤ 90 s in the previous study compared to 15-20 minutes in our study. As such, the duration of exposure and the time of testing (during exposure or post-exposure) may influence how intracortical circuits in the motor cortex are affected.

SICI and LICI have frequently been associated with GABAergic inhibitory neurons, where administration of drugs such as lorazepam and baclofen have confirmed that SICI is mediated by $GABA_A$ receptor activity (Di Lazzaro et al., 2000; Ziemann et al., 1996) and LICI is mediated by $GABA_B$ receptor activity (McDonnell, Orekhov, & Ziemann, 2006). Our findings indicate that extended exposure to cold water immersion stimuli may not only decrease $GABA_A$ receptor activity in the contralateral hemisphere, but also in the hemisphere ipsilateral to stimuli. In addition to activity changes in short interval inhibitory networks, facilitatory intracortical networks were altered in both hemispheres. ICF is principally mediated by excitatory glutamatergic interneurons and *N*-methyl-$_D$-aspartate (NMDA) receptors (Schwenkreis et al., 1999; Ziemann, Chen, et al., 1998), which suggests that extended cold water immersion of the forearm evoked bilateral increases in excitatory glutamatergic interneuron and NMDA receptor activity in the motor cortices.

Given that each M1 was affected by the intervention in the current study, there must be a mechanism where sensory input from one side of the body is distributed to both hemispheres. While the current study cannot reveal the exact neural structures involved, there is supporting evidence that the thalamus, anterior cingulate cortex, and amygdala played a role in our observed motor responses (Apkarian, Bushnell, Treede, & Zubieta, 2005; Apkarian et al., 2004). Most importantly, the thalamus is a relay centre for incoming nociception, is composed of the sensory discriminative and affective-motivational components of pain

(Cross, 1994), and has bilateral intra-thalamic projections (Crabtree & Isaac, 2002). Nociceptive neurons from the ventral posterolateral and ventral posteromedial thalamic nuclei primarily project to the primary somatosensory cortex and play an important role in discriminating stimuli (Andersson et al., 1997; Kenshalo & Isensee, 1983). In addition, the intralaminar and posterior aspect of ventromedial thalamic nuclei project to the somatosensory cortex and limbic structures (Shyu, Lin, Sun, Chen, & Chang, 2004), and are responsible for the affective-motivational aspect of pain (Craig, 2003). As the M1 receives ascending sensory inputs directly through the thalamus and indirectly through the somatosensory cortex, the thalamus is a likely mediator in distributing unilateral input to each hemisphere.

4.4. Cold water immersion of a single limb compromised reaction time of the opposite limb

The reaction time experiment provided further support that noxious sensation induced in a single limb causes widespread changes in the CNS. As already established, MEP amplitude responses to acute pain may increase or decrease depending on the intervention that is employed. In contrast, changes in temporal measures, such as MEP latency and the controlled reaction time task in experiment 3, tend to be optimal or sub-optimal in healthy individuals. Unlike the TMS derived measures, the outcomes of the voluntary movement task reflected cognitive processing involved with stimulus detection in addition to pain processing (Weiss, 1965). Given that MEP latency for the non-immersed limb was unaffected by cold water immersion, and reaction time for the non-immersed limb was slower with cold water, the single limb cold water immersion task appears to affect aspects of cognitive processing rather than the conduction velocity of motor pathways. This outcome is consistent with our paired-

pulse TMS findings, where the processing that occurs in neural networks is altered with single limb cold water immersion.

4.5. Considerations

Although the increase in MEP observed in the present study was attributed to changes in the M1, it cannot be overlooked that changes in neural activity occurred outside of the cerebral cortex, or even via enhanced muscle sympathetic nerve activity. The MEP is a summative measure of cortico-cortical, cortico-motoneuronal and spinal motoneurone synaptic excitability, so further research is required to examine the potential spinal and peripheral contributions to changes in MEP, SICI and ICF. A further consideration is that between-hemisphere differences were identified in intracortical circuits during the control condition. There would most likely be symmetry between hemispheres in the general population, however a small sample size was used in each experiment, and not every subject in experiment 1 participated in experiment 2. Given our within-subject design, group differences between hemispheres did not impact on the findings of the study. Finally, there was no assessment of reaction time on the immersed limb, because it has long been known that a cooled limb increases RT, whereby cooling of the limb and decreasing skin and tissue temperature reduces nerve conduction velocity in the sensory and motor nerves (Algafly & George, 2007; Herrera et al., 2010).

5. CONCLUSIONS

We have presented for the first time that immersion of a single limb in cold water caused an increase in amplitude for MEP and ICF, and a decrease in SICI, in the M1 of both

hemispheres. The activity occurring in the ipsilateral hemisphere due to the single limb cold water immersion also led to a functionally reduced reaction time for the non-immersed ECRB muscle during rapid wrist extension. These findings provide evidence that an intense sensory stimulus could lead to widespread activation of somatosensory systems, which in turn influence the intracortical motor circuitry in the contralateral hemisphere, as well as the ipsilateral hemisphere, to the limb exposed to the intervention.

ACKNOWLEDGMENTS

We would like to acknowledge Mr. Jacob Thorstensen and Mr. Daniel Mckeown for their assistance throughout data collection.

COMPETING INTERESTS

None of the authors have potential conflicts of interest to be disclosed. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHOR CONTRIBUTIONS

Experiments were performed in the Neural Control of Movement Laboratory at Griffith University. All authors designed the study protocol. E.T.D. and J.J.K. acquired the data. All authors analysed and interpreted the data and drafted or revised the final manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part

of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

REFERENCES

Algafly, A. A., & George, K. P. (2007). The effect of cryotherapy on nerve conduction velocity, pain threshold and pain tolerance. *Br J Sports Med, 41*(6), 365-369; discussion 369. doi:10.1136/bjsm.2006.031237

Andersson, J. L., Lilja, A., Hartvig, P., Langstrom, B., Gordh, T., Handwerker, H., & Torebjork, E. (1997). Somatotopic organization along the central sulcus, for pain localization in humans, as revealed by positron emission tomography. *Exp Brain Res, 117*(2), 192-199.

Apkarian, A. V., Bushnell, M. C., Treede, R. D., & Zubieta, J. K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *Eur J Pain, 9*(4), 463-484. doi:10.1016/j.ejpain.2004.11.001

Apkarian, A. V., Sosa, Y., Krauss, B. R., Thomas, P. S., Fredrickson, B. E., Levy, R. E., . . . Chialvo, D. R. (2004). Chronic pain patients are impaired on an emotional decision-making task. *Pain, 108*(1-2), 129-136. doi:10.1016/j.pain.2003.12.015

Brasil-Neto, J. P., Valls-Sole, J., Pascual-Leone, A., Cammarota, A., Amassian, V. E., Cracco, R., . . . Cohen, L. G. (1993). Rapid modulation of human cortical motor outputs following ischaemic nerve block. *Brain, 116 (Pt 3)*, 511-525.

Burns, E., Chipchase, L. S., & Schabrun, S. M. (2016). Reduced Short- and Long-Latency Afferent Inhibition Following Acute Muscle Pain: A Potential Role in the Recovery of Motor Output. *Pain Med*. doi:10.1093/pm/pnv104

Cain, D. M., Khasabov, S. G., & Simone, D. A. (2001). Response properties of mechanoreceptors and nociceptors in mouse glabrous skin: an in vivo study. *J Neurophysiol, 85*(4), 1561-1574. doi:10.1152/jn.2001.85.4.1561

Casey, K. L., Minoshima, S., Morrow, T. J., & Koeppe, R. A. (1996). Comparison of human cerebral activation pattern during cutaneous warmth, heat pain, and deep cold pain. *J Neurophysiol, 76*(1), 571-581. doi:10.1152/jn.1996.76.1.571

Coghill, R. C., Sang, C. N., Maisog, J. M., & Iadarola, M. J. (1999). Pain intensity processing within the human brain: a bilateral, distributed mechanism. *J Neurophysiol, 82*(4), 1934-1943. doi:10.1152/jn.1999.82.4.1934

Crabtree, J. W., & Isaac, J. T. (2002). New intrathalamic pathways allowing modality-related and cross-modality switching in the dorsal thalamus. *J Neurosci, 22*(19), 8754-8761.

Craig, A. D. (2003). A new view of pain as a homeostatic emotion. *Trends Neurosci, 26*(6), 303-307.

Cross, S. A. (1994). Pathophysiology of pain. *Mayo Clin Proc, 69*(4), 375-383.

Darian-Smith, I., Johnson, K. O., & Dykes, R. (1973). "Cold" fiber population innervating palmar and digital skin of the monkey: responses to cooling pulses. *J Neurophysiol, 36*(2), 325-346. doi:10.1152/jn.1973.36.2.325

Di Lazzaro, V., Oliviero, A., Meglio, M., Cioni, B., Tamburrini, G., Tonali, P., & Rothwell, J. C. (2000). Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. *Clin Neurophysiol, 111*(5), 794-799.

Fagius, J., Karhuvaara, S., & Sundlof, G. (1989). The cold pressor test: effects on sympathetic nerve activity in human muscle and skin nerve fascicles. *Acta Physiol Scand, 137*(3), 325-334. doi:10.1111/j.1748-1716.1989.tb08760.x

Fierro, B., De Tommaso, M., Giglia, F., Giglia, G., Palermo, A., & Brighina, F. (2010). Repetitive transcranial magnetic stimulation (rTMS) of the dorsolateral prefrontal cortex (DLPFC) during capsaicin-induced pain: modulatory effects on motor cortex excitability. *Exp Brain Res, 203*(1), 31-38. doi:10.1007/s00221-010-2206-6

Henderson, L. A., Bandler, R., Gandevia, S. C., & Macefield, V. G. (2006). Distinct forebrain activity patterns during deep versus superficial pain. *Pain, 120*(3), 286-296. doi:10.1016/j.pain.2005.11.003

Herrera, E., Sandoval, M. C., Camargo, D. M., & Salvini, T. F. (2010). Motor and sensory nerve conduction are affected differently by ice pack, ice massage, and cold water immersion. *Phys Ther, 90*(4), 581-591. doi:10.2522/ptj.20090131

Hess, C. W., Mills, K. R., & Murray, N. M. (1987). Responses in small hand muscles from magnetic stimulation of the human brain. *J Physiol, 388*, 397-419.

Hodges, P. W., & Bui, B. H. (1996). A comparison of computer-based methods for the determination of onset of muscle contraction using electromyography. *Electroencephalogr Clin Neurophysiol, 101*(6), 511-519.

Kavanagh, J. J., Bisset, L. M., & Tsao, H. (2012). Deficits in reaction time due to increased motor time of peroneus longus in people with chronic ankle instability. *J Biomech, 45*(3), 605-608. doi:10.1016/j.jbiomech.2011.11.056

Kenshalo, D. R., Jr., & Isensee, O. (1983). Responses of primate SI cortical neurons to noxious stimuli. *J Neurophysiol, 50*(6), 1479-1496. doi:10.1152/jn.1983.50.6.1479

Koltzenburg, M., Stucky, C. L., & Lewin, G. R. (1997). Receptive properties of mouse sensory neurons innervating hairy skin. *J Neurophysiol, 78*(4), 1841-1850. doi:10.1152/jn.1997.78.4.1841

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., . . . Marsden, C. D. (1993). Corticocortical inhibition in human motor cortex. *J Physiol, 471*, 501-519.

Le Pera, D., Graven-Nielsen, T., Valeriani, M., Oliviero, A., Di Lazzaro, V., Tonali, P. A., & Arendt-Nielsen, L. (2001). Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain. *Clin Neurophysiol, 112*(9), 1633-1641.

Loggia, M. L., Edwards, R. R., Kim, J., Vangel, M. G., Wasan, A. D., Gollub, R. L., . . . Napadow, V. (2012). Disentangling linear and nonlinear brain responses to evoked deep tissue pain. *Pain, 153*(10), 2140-2151. doi:10.1016/j.pain.2012.07.014

Macefield, V. G., Gandevia, S. C., & Henderson, L. A. (2007). Discrete changes in cortical activation during experimentally induced referred muscle pain: a single-trial fMRI study. *Cereb Cortex, 17*(9), 2050-2059. doi:10.1093/cercor/bhl113

Maeda, L., Ono, M., Koyama, T., Oshiro, Y., Sumitani, M., Mashimo, T., & Shibata, M. (2011). Human brain activity associated with painful mechanical stimulation to muscle and bone. *J Anesth, 25*(4), 523-530. doi:10.1007/s00540-011-1173-9

Martin, P. G., Weerakkody, N., Gandevia, S. C., & Taylor, J. L. (2008). Group III and IV muscle afferents differentially affect the motor cortex and motoneurones in humans. *J Physiol, 586*(5), 1277-1289. doi:10.1113/jphysiol.2007.140426

McDonnell, M. N., Orekhov, Y., & Ziemann, U. (2006). The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Exp Brain Res, 173*(1), 86-93. doi:10.1007/s00221-006-0365-2

Mercier, C., Gagne, M., Reilly, K. T., & Bouyer, L. J. (2016). Effect of Experimental Cutaneous Hand Pain on Corticospinal Excitability and Short Afferent Inhibition. *Brain Sci, 6*(4). doi:10.3390/brainsci6040045

Nash, P. G., Macefield, V. G., Klineberg, I. J., Gustin, S. M., Murray, G. M., & Henderson, L. A. (2010a). Bilateral activation of the trigeminothalamic tract by acute orofacial cutaneous and muscle pain in humans. *Pain, 151*(2), 384-393. doi:10.1016/j.pain.2010.07.027

Nash, P. G., Macefield, V. G., Klineberg, I. J., Gustin, S. M., Murray, G. M., & Henderson, L. A. (2010b). Changes in human primary motor cortex activity during acute cutaneous and muscle orofacial pain. *J Orofac Pain, 24*(4), 379-390.

Niddam, D. M., Yeh, T. C., Wu, Y. T., Lee, P. L., Ho, L. T., Arendt-Nielsen, L., . . . Hsieh, J. C. (2002). Event-related functional MRI study on central representation of acute muscle pain induced by electrical stimulation. *Neuroimage, 17*(3), 1437-1450.

Peyron, R., Garcia-Larrea, L., Gregoire, M. C., Costes, N., Convers, P., Lavenne, F., . . . Laurent, B. (1999). Haemodynamic brain responses to acute pain in humans: sensory and attentional networks. *Brain, 122 (Pt 9)*, 1765-1780.

Ringkamp, M., Peng, Y. B., Wu, G., Hartke, T. V., Campbell, J. N., & Meyer, R. A. (2001). Capsaicin responses in heat-sensitive and heat-insensitive A-fiber nociceptors. *J Neurosci, 21*(12), 4460-4468.

Salo, K. S., Vaalto, S. M. I., Koponen, L. M., Nieminen, J. O., & Ilmoniemi, R. J. (2019). The effect of experimental pain on short-interval intracortical inhibition with multi-locus transcranial magnetic stimulation. *Exp Brain Res, 237*(6), 1503-1510. doi:10.1007/s00221-019-05502-5

Schabrun, S. M., & Hodges, P. W. (2012). Muscle pain differentially modulates short interval intracortical inhibition and intracortical facilitation in primary motor cortex. *J Pain, 13*(2), 187-194. doi:10.1016/j.jpain.2011.10.013

Schabrun, S. M., Jones, E., Kloster, J., & Hodges, P. W. (2013). Temporal association between changes in primary sensory cortex and corticomotor output during muscle pain. *Neuroscience, 235*, 159-164. doi:10.1016/j.neuroscience.2012.12.072

Schepers, R. J., & Ringkamp, M. (2010). Thermoreceptors and thermosensitive afferents. *Neurosci Biobehav Rev, 34*(2), 177-184. doi:10.1016/j.neubiorev.2009.10.003

Schmelz, M., Schmid, R., Handwerker, H. O., & Torebjork, H. E. (2000). Encoding of burning pain from capsaicin-treated human skin in two categories of unmyelinated nerve fibres. *Brain, 123 Pt 3*, 560-571.

Schwenkreis, P., Witscher, K., Janssen, F., Addo, A., Dertwinkel, R., Zenz, M., . . . Tegenthoff, M. (1999). Influence of the N-methyl-D-aspartate antagonist memantine on human motor cortex excitability. *Neurosci Lett, 270*(3), 137-140.

Shyu, B. C., Lin, C. Y., Sun, J. J., Chen, S. L., & Chang, C. (2004). BOLD response to direct thalamic stimulation reveals a functional connection between the medial thalamus and the anterior cingulate cortex in the rat. *Magn Reson Med, 52*(1), 47-55. doi:10.1002/mrm.20111

Svensson, P., Miles, T. S., McKay, D., & Ridding, M. C. (2003). Suppression of motor evoked potentials in a hand muscle following prolonged painful stimulation. *Eur J Pain, 7*(1), 55-62.

Takahashi, K., Taguchi, T., Tanaka, S., Sadato, N., Qiu, Y., Kakigi, R., & Mizumura, K. (2011). Painful muscle stimulation preferentially activates emotion-related brain regions compared to painful skin stimulation. *Neurosci Res, 70*(3), 285-293. doi:10.1016/j.neures.2011.04.001

Uematsu, H., Shibata, M., Miyauchi, S., & Mashimo, T. (2011). Brain imaging of mechanically induced muscle versus cutaneous pain. *Neurosci Res, 70*(1), 78-84. doi:10.1016/j.neures.2011.01.015

Valeriani, M., Restuccia, D., Di Lazzaro, V., Oliviero, A., Le Pera, D., Profice, P., . . . Tonali, P. (2001). Inhibition of biceps brachii muscle motor area by painful heat stimulation of the skin. *Exp Brain Res, 139*(2), 168-172.

Valls-Sole, J., Pascual-Leone, A., Wassermann, E. M., & Hallett, M. (1992). Human motor evoked responses to paired transcranial magnetic stimuli. *Electroencephalogr Clin Neurophysiol, 85*(6), 355-364.

Weiss, A. D. (1965). The Locus of Reaction Time Change with Set, Motivation, and Age. *J Gerontol, 20*, 60-64.

Yamamoto, K., Iwase, S., & Mano, T. (1992). Responses of muscle sympathetic nerve activity and cardiac output to the cold pressor test. *Jpn J Physiol, 42*(2), 239-252.

Ziemann, U. (2001). Sensory-motor integration in human motor cortex at the pre-motoneurone level: beyond the age of simple MEP measurements. *J Physiol, 534*(Pt 3), 625.

Ziemann, U., Chen, R., Cohen, L. G., & Hallett, M. (1998). Dextromethorphan decreases the excitability of the human motor cortex. *Neurology, 51*(5), 1320-1324.

Ziemann, U., Corwell, B., & Cohen, L. G. (1998). Modulation of plasticity in human motor cortex after forearm ischemic nerve block. *J Neurosci, 18*(3), 1115-1123.

Ziemann, U., Lonnecker, S., Steinhoff, B. J., & Paulus, W. (1996). The effect of lorazepam on the motor cortical excitability in man. *Exp Brain Res, 109*(1), 127-135.

Table 1. Resting motor threshold (rMT) and resting ECRB EMG characteristics for the control condition and the single limb cold water immersion condition.

rMT (% stimulator output)	**Contralateral M1**	**Ipsilateral M1**
Cold water immersion condition	46 ± 5	43 ± 7
Control condition	47 ± 5	43 ± 5

ECRB EMG rms amplitude (μV)	**Immersed limb**	**Non-immersed limb**
Cold water immersion condition	46.99 ± 20.13	23.57 ± 21.18
Control condition	39.46 ± 13.65	20.70 ± 18.54

N.b. Data are presented as means ± SD. For all cold water immersion data, a single limb was immersed in a water bath. The control condition assessed the same hemisphere and/or limb, but without a limb being placed in water.

Figure 1. Experiment design and participant setup. Three experiments were performed where a single limb was immersed in cold water for each experiment, so that only the hand and forearm were immersed. The control condition had the same setup for each experiment, however there was no water in the bath. ECRB was the target muscle in each experiment. Experiment 1 assessed resting motor threshold (rMT), motor evoked potential (MEP) amplitude, short interval intracortical inhibition (SICI), long interval intracortical inhibition (LICI) and intracortical facilitation (ICF) of the contralateral primary motor cortex (M1). Experiment 2 repeated these measurements for the ipsilateral M1. Experiment 3 assessed

reaction time of the opposite limb via rapid wrist extension. The order of testing (control vs intervention) was counter-balanced where half of the participants performed the control condition first. Perceptions of pain and temperature (†) were collected during the acclimation period of the single limb cold water immersion condition and immediately following the termination of testing during both experiments.

Figure 2. Measures of pain and temperature sensation intensity (11-point NRS) during the acclimation period of single limb cold water immersion in experiment 1. Both pain and temperature intensity were significantly greater than the reference value of 0, which was reported during the control condition, across all time intervals. Data is presented as group means ± SD. Asterisks indicates significant differences between time intervals ($p < 0.05$).

Figure 3. Change in MEP amplitude (A) and MEP latency (B) of the baseline MEPs for the relaxed ECRB muscle during the control and cold water immersion conditions obtained from the contralateral and ipsilateral M1. MEP amplitude was measured peak-to-peak (mV), and MEP latency was the duration (ms) from test stimulus (TS) to the first negative deflection of the MEP. Data is presented as group means ± SD. Asterisks indicates a significant difference between control and cold water immersion conditions ($p < 0.05$).

Figure 4. Conditioned MEP amplitude for (A) short interval intracortical inhibition (SICI), (B) intracortical facilitation (ICF), and (C) long interval intracortical inhibition (LICI) for the contralateral and ipsilateral hemispheres. Data are presented for the control and the single limb cold water immersion conditions. The size of the conditioned MEP for SICI, ICF and

LICI are expressed as a percentage of the baseline unconditioned MEP for the respective test stimuli. Data are presented as group means ± SD. Asterisk (*) indicates a significant difference between the control and single limb cold water immersion condition, and asterisks (**) indicates a significant difference between the contralateral and ipsilateral hemispheres ($p < 0.05$).

Figure 5. ECRB muscle reaction times (ms) when performing rapid wrist extension in response to an auditory tone. Reaction time is presented for non-immersed limb during cold water immersions, as well as for the same limb during control trials. Box plots illustrate the 5th, 25th, 50th (median), 75th, and 95th percentile. Values above the 95th and below the 5th percentile are plotted as points. Asterisks indicates a significant difference between the control and cold water immersion condition ($p < 0.05$).