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Excitability of intracortical inhibitory and facilitatory circuits during ischemic nerve block.

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Abstract

Purpose: The primary motor cortex is capable of rapid, reversible plastic changes and longer-term, more permanent reorganization. Ischemic nerve block (INB) is a model of deafferentation-induced short-term plasticity. We used transcranial magnetic stimulation to examine whether changes in the excitability of short- and/or long-interval intracortical inhibitory (SICI, LICI) or short-interval intracortical facilitatory (SICF) circuits underlie the corticospinal excitability increases observed during INB.

Methods: SICI and LICI recruitment curves, obtained by varying conditioning stimulus intensity, and SICF were measured at multiple inter-stimulus intervals (ISIs).

Results: Forearm flexor MEP amplitude increased during INB at the wrist; this was not accompanied by changes in SICI at ISIs of 1 or 2 ms, in SICF at ISIs of 1.2, 2.7, or 4.4 ms, or in LICI at an ISI of 80 ms, but was accompanied by an increase in LICI at an ISI of 150 ms.

Conclusions: The results suggest that (1) the increased excitability of forearm flexors is not due to reduced SICI or LICI or increased SICF, and (2) LICI measured at ISIs of 80 and 150 ms are distinct processes. We discuss the importance of identifying distinct processes of LICI and speculate regarding other mechanisms that could potentially underlie INB-induced plasticity.

Keywords: ischemic nerve block, transcranial magnetic stimulation, short-interval intracortical inhibition, long-interval intracortical inhibition, short-interval intracortical facilitation, primary motor cortex, cortical plasticity.

1. Introduction

It is well established that the human primary motor cortex (M1) is capable of both rapid, reversible plastic changes and longer-term, more permanent reorganization. During ischemic nerve block (INB) a tourniquet placed around the limb restricts blood flow to the body part distal to the tourniquet; this blood flow restriction impedes transmission of efferent and afferent information to and from the limb and results in temporary paralysis and numbness. Transcranial magnetic stimulation (TMS) studies show that muscles proximal to an INB have increased motor evoked potential (MEP) amplitudes (Brasil-Neto et al., 1992; Brasil-Neto et al., 1993; McNulty et al. 2002; Ridding and Rothwell, 1995; Ridding and Rothwell, 1997; Ziemann et al., 1998a), steeper input/output (I/O) curve slopes (Ridding and Rothwell, 1995), and larger motor representation maps (Ridding and Rothwell, 1995; Ridding and Rothwell, 1997). It is generally assumed that INB-induced changes in MEP amplitude have a cortical origin as Brasil-Neto and colleagues (1993) showed that the amplitude of MEPs elicited by either transcranial electrical stimulation or spinal electrical stimulation, and of H-reflexes elicited by peripheral nerve stimulation, did not change in the presence of an INB. Thus, INB, which is an established model of deafferentation-induced short-term plasticity in the human motor cortex (Brasil-Neto et al., 1992; Brasil-Neto et al., 1993; McNulty et al., 2002; Ridding and Rothwell, 1995; Ridding and Rothwell, 1997; Ziemann et al., 1998a; Ziemann et al., 2002a; Ziemann et al., 2002b), provides one means by which to investigate the mechanisms underlying short-term plastic changes in the adult motor cortex.

Increases in corticospinal excitability have been reported in muscles proximal to an INB within seven to eight minutes following application of the tourniquet (Brasil-Neto et al., 1993). The rapid nature of these changes suggests that disinhibition of existing cortical circuits might play an important role, and it has been hypothesised that in the case of INB this

might be in the form of a release of intracortical inhibition (Cohen et al., 1993; Jacobs and Donoghue, 1991).

Paired-pulse TMS can be used to measure the excitability of two types of intracortical inhibitory circuits. When a subthreshold conditioning stimulus (S1) precedes a suprathreshold test stimulus (S2) by 1-6 ms the amplitude of the MEP elicited by S2 is suppressed due to activation of short-interval intracortical inhibitory (SICI) circuits (Kujirai et al., 1993). Similarly, when a suprathreshold S1 precedes a second suprathreshold S2 by ~50-200 ms the amplitude of the MEP elicited by S2 is suppressed due to activation of long-interval intracortical inhibitory (LICI) circuits (Valls-Solé et al., 1992; Wassermann et al., 1996). Ziemann and colleagues (1998a) investigated activity in one of these two intracortical inhibitory circuits (SICI) during INB and found that a tourniquet placed across the elbow increased corticospinal excitability in biceps but did not change the excitability of SICI circuits. However, SICI might still play a role in INB-induced corticospinal excitability increases as these authors only measured SICI at a single S1 intensity and at inter-stimulus intervals (ISIs) of 2 and 4 ms. Both SICI and LICI are sensitive to S1 intensity, resulting in U-shaped curves (when the amount of inhibition is plotted as a function of increasing S1 intensity; Chen et al., 1998; Hammond and Garvey, 2006; Ilic et al., 2002; Kujirai et al., 1993). The descending limbs of SICI and LICI recruitment curves, where inhibition increases with increasing S1 intensity, represent the progressive recruitment of populations of inhibitory interneurons that mediate these processes (Peurala et al., 2008). Further evidence that several neural populations contribute to these processes comes from studies showing that SICI has at least two distinct phases; an early phase measured with an ISI of 1 ms and a later phase measured with ISIs longer than 2 ms (Fisher et al., 2002; Hanajima et al., 2003; Ortu et al., 2008; Roshan et al., 2003; Vucic et al., 2009). It remains possible therefore, that

modulations in SICI (and/or LICI) during INB underlie the increased excitability observed in muscles proximal to an INB, but that these modulations are apparent at higher or lower S1 intensities, or at different ISIs, than previously tested.

While it is generally thought that reduced activity of inhibitory circuits underlies the rapid changes observed after inflation of a tourniquet, the increase in corticospinal excitability evident during INB could also be due to an increase in the activity of intracortical facilitatory circuits. Like SICI and LICI, SICF can be measured with paired-pulse TMS. When a suprathreshold S1 precedes a subthreshold S2 at an indirect-wave (I-wave) interval (multiples of ~1.5 ms) the amplitude of the MEP is larger than that elicited by a single suprathreshold stimulus (Ziemann et al., 1998b). SICF has three prominent early peaks; the first and strongest occurs at inter-stimulus intervals (ISIs) of 1.1-1.5 ms, the second at ISIs of 2.3-2.9 ms, and the third at ISIs of 4.1-4.4 ms (Ziemann et al., 1998b). The facilitatory effect of paired-pulses delivered at these precise intervals is thought to result from I-wave facilitation (Hanajima et al., 2002; Ortu et al., 2008; Ziemann et al., 1998b; Ziemann et al., 1998c).

Here we investigated whether a release of intracortical inhibition or an increase in intracortical facilitation in M1 can explain the increase in corticospinal excitability observed during INB. Single-pulse TMS was used to examine changes in corticospinal excitability and paired-pulse TMS was used to examine SICI, LICI, and SICF acting on forearm flexors proximal to a tourniquet placed around the wrist. Recruitment curves for SICI and LICI were obtained by varying S1 intensities at two ISIs (SICI: 1 and 2 ms; LICI: 80 and 150 ms). In order to investigate potential changes at the peaks of I-wave facilitation during INB SICF was measured at three ISIs (1.2, 2.7, 4.4 ms). We hypothesised that if these intracortical processes play a role in the increase in corticospinal excitability observed during INB we would

observe a decrease in the excitability of SICI and/or LICI circuits and/or an increase in the excitability of SICF circuits acting on the forearm flexors.

2. *Methods*

2.1 Subjects

Experiment 1 measured SICI at a 2-ms ISI ($SICI_2$) and LICI at an 80-ms ISI ($LICI_{80}$) in 11 healthy adults (eight females) with age ranging from 21 to 35 years (median age 24 years); Experiment 2 measured SICI at a 1-ms ISI ($SICI_1$) and LICI at a 150-ms ISI ($LICI_{150}$) in 11 healthy adults (5 females) with age ranging from 19 to 36 years (median age 24 years); and Experiment 3 measured SICF in 12 healthy adults (six females) with age ranging from 19 to 29 years (median age 25 years). All samples were completely independent. The protocol was in accordance with the Declaration of Helsinki and was approved by The University of Western Australia Human Research Ethics Committee. All subjects gave written informed consent prior to testing.

2.2 Ischemic Nerve Block

Subjects sat comfortably in a slightly reclined position with their neck and head supported and their right forearm resting comfortably on a table in a semi-pronated position with their wrist in a neutral position and their elbow slightly flexed. The forearm, wrist, and hand were stabilised using a vacuum cast (a sealed plastic bag filled with polystyrene balls) which was first molded around the arm and then had the air extracted to secure the arm in position. A pediatric blood pressure cuff was placed around the forearm, positioned immediately proximal to the wrist and inflated for a predetermined time of 30 minutes in Experiments 1 and 2, and 15 minutes in Experiment 3. We chose to reduce the cuff inflation time in Experiment 3 because previous experiments in our laboratory, and the results of Brasil-Neto

and colleagues (1993), show that significant increases in corticospinal excitability occur within 15 minutes. Even though INB is a safe and widely-used procedure we did not want to inflate the cuff longer than necessary. During INB the fingers adopted a slightly flexed posture as the ischemia induced a gradual paralysis of the intrinsic hand muscles which are essential for maintaining extension at the interphalangeal joints. None of the participants reported significant discomfort and all of the measures were taken before the cuff was deflated (the measures obtained 30 minutes following cuff inflation in Experiments 1 and 2 took approximately 10 minutes in total (so participants had 30 minutes during which there was no TMS between measurement times) and the measures obtained 15 minutes following cuff inflation in Experiment 3 took approximately 5 minutes in total (participants had 15 minutes during which they received no TMS).

2.3 Transcranial Magnetic Stimulation

Electromyographic (EMG) activity was recorded from the relaxed forearm flexors (primarily flexor carpi radialis; FCR) with Ag-AgCl electrodes placed in a belly-tendon configuration. The EMG signal was amplified (1000x), band pass filtered (10-1000 Hz), and digitized at 4000 Hz for 200 ms beginning 50 ms before delivery of the first TMS pulse (S1). Two Magstim 200 stimulators connected through a BiStim module generated single- and paired-pulse stimuli, and all stimuli were delivered through a figure-of-eight coil (90 mm diameter) placed tangentially to the scalp with the handle pointing backward and at a 45° angle away from the midline. The optimal stimulation site was identified as the site at which five successive suprathreshold pulses produced the largest mean MEP amplitude in FCR. Resting motor threshold (rMT) was calculated at the optimal stimulation site and was defined as the minimum stimulus intensity, to the nearest 1% of the stimulator output, required to elicit

MEPs in FCR greater than 50 µV in at least five of ten successive single TMS pulses (Rossini et al., 1999).

2.4 Procedure

2.4.1   Experiments 1 and 2: SICI and LICI Recruitment Curves

The procedure for Experiments 1 and 2 was identical except for the ISIs at which we tested SICI and LICI; in Experiment 1 we used ISIs of 2 ms ($SICI_2$) and 80 ms ($LICI_{80}$) and in Experiment 2 we used ISIs of 1 ms ($SICI_1$) and 150 ms ($LICI_{150}$). Single-pulse MEP amplitudes and SICI and LICI recruitment curves were obtained before cuff inflation (baseline) and 30 minutes following cuff inflation (T30). S1 intensities were set as a percentage of rMT and S2 intensity was set as the intensity that elicited an MEP of approximately 1 mV ($SI_{1\ mV}$) in FCR. This S2 intensity was chosen as previous studies have shown that maximum inhibition occurs when S2 is set to $SI_{1\ mV}$ (Ilic et al., 2002; Sanger et al., 2001).

SICI recruitment curves were obtained from eight blocks of six trials: one single-pulse TMS trial and five paired-pulse trials with S1 intensities of 40, 50, 60, 70, and 80% of rMT, S2 intensity of $SI_{1\ mV}$, and an ISI of 2 ms in Experiment 1 ($SICI_2$) and 1 ms in Experiment 2 ($SICI_1$). LICI recruitment curves were obtained from eight blocks of five trials: one single-pulse TMS trial and four paired–pulse TMS trials with S1 intensities of 110, 120, 130 and 140% of rMT, S2 intensity of $SI_{1\ mV}$, and an ISI of 80 ms in Experiment 1 ($LICI_{80}$) and 150 ms in Experiment 2 ($LICI_{150}$). In both experiments SICI and LICI recruitment curves were measured twice; once at baseline and again at T30 (30 minutes following cuff inflation). In Experiment 1, $SICI_2$ recruitment curves were obtained before $LICI_{80}$ recruitment curves for each participant and in Experiment 2, the order of $SICI_1$ and $LICI_{150}$ recruitment curves was

counterbalanced. For both Experiments 1 and 2 the stimulation intensities used for both SICI and LICI recruitment curves were determined at baseline (before cuff inflation) and did not change throughout the experiment.

It is worth noting here that although the increase in corticospinal excitability induced by the block increased the amplitude of MEPs evoked by a stimulus intensity of $SI_{1\ mV}$ determined at baseline, we chose not to alter the S2 intensity for our paired-pulse measurements at T30 because previous research has shown that SICI measurements are unaffected by S2 amplitude increases as large as 300% (Garry and Thomson, 2009; Ridding et al., 1995; Sailer et al., 2002; Sanger et al., 2001). In contrast, LICI is sensitive to S2 intensity, decreasing as S2 intensity (and thus MEP amplitude) increase, but this has only been demonstrated by increasing S2 from an intensity that elicits an MEP of ~1 mV to an intensity that elicits an MEP of ~4 mV (Sanger et al., 2001; Sailer et al., 2002). Based on previous studies in our laboratory and published work examining MEP amplitude during INB, we expected the S2 intensity that elicited MEPs of ~1 mV at baseline to elicit MEPs of ~1.5-2 mV during INB. This is well below the 400% increase found to affect LICI and well within the limits of those increases that do not affect SICI. We also chose not to alter the S1 intensity for our paired-pulse measurements because previous research has shown that the rMT of muscles proximal to a tourniquet does not change during INB (Ridding and Rothwell, 1997; Ziemann et al., 1998a). Since both SICI and LICI change as a function of S1 intensity, we tested a range of S1 intensities at baseline and T30 (the intensities used at both times points were fixed proportions of rMT measured at baseline).

2.4.2 *Experiment 3: SICF*

Single-pulse MEP amplitudes and SICF were obtained before cuff inflation (baseline) and 15 minutes following cuff inflation (T15). S1 was set at $SI_{1\ mV}$ for FCR and S2 was set at 90% of

rMT for FCR, consistent with the conventional protocol for measuring SICF (Ziemann et al., 1998b). SICF was measured at three ISIs, 1.2, 2.7, and 4.4 ms. MEP amplitudes were obtained by randomly presenting ten single- and ten paired-pulse TMS trials at each ISI at baseline and T15 (15 minutes following cuff inflation). The stimulation intensities were determined at baseline (before cuff inflation) and did not change for the second measurement.

2.5 Data Analysis

The peak-to-peak amplitude of FCR MEPs (in mV) was obtained from 40 ms of EMG activity beginning 10 ms after S2 for SICI and SICF measures and 10 ms after both S1 and S2 for LICI measures. In Experiments 1 and 2, corticospinal excitability was measured by single-pulse MEP amplitudes; each subject's mean MEP amplitude obtained from all 16 single test pulses (taken from both the SICI and LICI curve measurement blocks) at T30 was expressed as a ratio of that subject's single-pulse mean MEP amplitude obtained at baseline. Ratios were log-transformed to normalize the distribution prior to analysis and a one-sample *t*-test was performed to compare the single-pulse MEP amplitude ratios to zero (baseline). SICI and LICI were quantified by expressing the mean paired pulse MEP amplitude for a given S1 intensity as a ratio of the mean single pulse MEP amplitude. Ratios were calculated for each S1 intensity at baseline and at T30. Ratios were log-transformed to normalize the distributions prior to analysis and repeated-measures analyses of variance (ANOVA) were used to assess whether SICI and LICI recruitment curves changed from baseline to 30 minutes following the inflation of the cuff (Phase). Separate analyses were performed for each ISI. Because we were interested in the emergence of SICI and LICI we only examined those S1 intensities that make up the descending limb of the recruitment curves; as a result, the recruitment curves were linear and we fitted them with linear regressions to obtain the

slope of each individual curve. Paired-samples t-tests were performed on the slopes at baseline and T30.

In Experiment 3, as in Experiments 1 and 2, corticospinal excitability was measured by single pulse MEP amplitudes; each subject's mean MEP amplitude obtained from single test pulses at T15 was expressed as a ratio of that subject's single-pulse mean MEP amplitude obtained at baseline. Ratios were log-transformed to normalize the distribution prior to analysis and a one-sample t-test was performed to compare the single-pulse MEP amplitude ratios to zero (baseline). SICF was quantified as the mean paired-pulse MEP amplitude expressed as a ratio of the mean single-pulse MEP amplitude. Ratios were calculated separately for each ISI at baseline and T15. Ratios were log-transformed to normalize the distributions prior to analysis. One-sample t-tests were performed to compare the SICF ratios at each ISI to zero (no facilitation) and a repeated-measures ANOVA was used to compare changes in SICF from baseline to T15.

The mean was used as the measure of central tendency in all analyses. Back transformed means of all log transformed ratios and standard errors are reported. In Experiment 2 ($SICI_1$ and $LICI_{150}$), one participant was excluded from the $SICI_1$ analysis and another participant was excluded from the $LICI_{150}$ analysis due to technical problems.

3. Results

3.1 Experiment 1 and 2: SICI and LICI Recruitment Curves

The mean rMT was 50% and 46% of maximum stimulator output for Experiments 1 and 2 respectively and the corresponding mean SI_{1mV} intensities were 60% and 56% of maximum stimulator output (the mean MEP amplitude at baseline was 1.0 mV and 0.8 mV for

Experiments 1 and 2 respectively). In Experiment 1, the mean MEP amplitude increased from 1.0 mV at baseline to 1.7 mV 30 minutes following inflation of the tourniquet; the back-transformed ratios showed that the mean MEP amplitude increased by 55% from baseline to T30 ($t(10)=3.0$, $P<.05$). In Experiment 2, the mean MEP amplitude increased from 0.8 mV at baseline to 1.2 mV 30 minutes following inflation of the tourniquet; the back-transformed ratios showed that the mean MEP amplitude increased by 49 % from baseline to T30 ($t(10)=2.4$, $P<.05$). Fig. 1 shows SICI recruitment curves from FCR in Experiments 1 and 2. Both $SICI_1$ and $SICI_2$ in FCR increased with increasing S1 intensity. The similarity of the SICI curves obtained at baseline and T30 in both experiments shows that despite the substantial increase in MEP amplitude following 30 minutes of INB there was no decrease in either $SICI_1$ or $SICI_2$ at any of the S1 intensities. There was no effect of Phase (Baseline, T30) for either $SICI_1$ ($F(1,9)=0.6$, $P>.05$) or $SICI_2$ recruitment curves ($F(1,10)=0.4$, $P>.05$) and no interaction of Phase and S1 Intensity for either $SICI_1$ ($F(4,36)=0.5$, $P>.05$) or $SICI_2$ ($F(4,40)=1.3$, $P>.05$). Furthermore, the slopes of individual subject recruitment curves did not differ at baseline and T30 for either $SICI_1$ (Baseline: -1.38, T30: -1.45, $t(9)=0.4$, $P>.05$) or $SICI_2$ (Baseline: -1.36, T30: -1.04, $t(10)=1.9$, $P>.05$).

Fig. 2 shows LICI recruitment curves from FCR in Experiments 1 and 2. Both $LICI_{80}$ and $LICI_{150}$ in FCR increased with increasing S1 intensity. The similarity of the $LICI_{80}$ curves obtained at baseline and T30 (left panel) shows that despite the substantial increase in MEP amplitude following 30 minutes of INB there was no decrease in $LICI_{80}$ at any of the S1 intensities tested; the repeated measures ANOVA showed no main effect of Phase ($F(1,10)=0.5$, $P>.05$) and no interaction of Phase and S1 Intensity for $LICI_{80}$ ($F(3,30)=1.6$, $P>.05$). Furthermore, the slope of individual subject $LICI_{80}$ recruitment curves did not differ at baseline and T30 (Baseline: -1.09, T30: -1.66, $t(10)=1.5$, $P>.05$). The right panel of Fig. 2,

however, shows that $LICI_{150}$ *increased* from baseline to T30; there was a significant main effect of Phase ($F(1, 9) = 7.9$, $P<.05$) and a significant interaction of Phase and S1 Intensity ($F(3,27)=6.5$, $P<.05$), which was due to significantly greater $LICI_{150}$ at the higher two S1 intensities at T30 than at baseline (both $t(9)>2.6$, $P<.05$). Furthermore, the slope of individual subject $LICI_{150}$ recruitment curves was greater at T30 than at baseline (Baseline: -1.15, T30: -1.90, $t(9)=2.6$, $P<.05$). These results show a differential modulation of $LICI_{80}$ and $LICI_{150}$ during INB with no change in $LICI_{80}$ and a significant increase (not the hypothesised decrease) in $LICI_{150}$.

3.2 Experiment 3: SICF

The mean rMT was 49% of maximum stimulator output and the mean SI_{1mV} intensity was 58% of maximum stimulator output (the mean MEP amplitude at baseline was 1.2 mV). The mean MEP amplitude increased from 1.2 mV at baseline to 1.7 mV 15 minutes following inflation of the tourniquet; the back-transformed ratios showed that the mean MEP amplitude increased by 46% from baseline to T15 ($t(11)=3.6$, $P<.05$). Fig. 3 shows SICF at each ISI at baseline and T15. As expected, SICF declined systematically with increasing ISI; the repeated measures ANOVA showed a significant main effect of ISI ($F(2,22)=8.9$, $P<.05$). SICF was present at the first two ISIs at baseline and T15 (all $t(11)>2.9$, $P<.05$), but not the third ISI at either baseline or T15 (both $t(11)<1.5$, $P>.05$). Despite the substantial increase in MEP amplitude 15 minutes following cuff inflation, the similarity of SICF at each of the three ISIs at baseline and T15 shows that there was no systematic increase in SICF at any of the ISIs; there was no effect of Phase (Baseline, T15: $F(1,11)=0.5$, $P>.05$) and no interaction of Phase and ISI ($F(2,22)=0.6$, $P>.05$).

4. Discussion

In the current study we examined changes in corticospinal excitability and the excitability of SICI, LICI, and SICF circuits of forearm flexors following inflation of a tourniquet at the wrist. We found that INB at the wrist substantially increased the corticospinal excitability of forearm flexors, but that this was not accompanied by a reduction in SICI or LICI (tested at two ISIs and at a range of S1 intensities), nor by any increase in SICF at the three ISIs tested. Indeed, the only systematic change we observed following cuff inflation was an increase in LICI at an ISI of 150 ms measured 30 minutes after cuff inflation. Paradoxically, this increase in intracortical inhibition was accompanied by an increase in corticospinal excitability of approximately 50%.

There are three main findings from the current study: first, a reduction in neither SICI nor LICI can explain the increase in excitability of the forearm flexors evident during INB at the wrist; second, $LICI_{80}$ and $LICI_{150}$ were differentially affected by INB, thus providing evidence for distinct LICI processes at these ISIs; and third, an increase in SICF cannot explain the increase in excitability of the forearm flexors evident during INB.

The current SICI results complement and extend those of Ziemann and colleagues (1998a), who showed no INB-induced change in the late phase of SICI (ISIs of 2 and 4 ms) measured with a single S1 intensity, by demonstrating that neither $SICI_1$ nor $SICI_2$, tested over a wide range of S1 intensities, mediates the increase in corticospinal excitability observed in muscles proximal to an INB. Previous studies have shown that there is no correlation between SICI at 1 ms and SICI at 2 or 3 ms, suggesting different circuits mediate these two phases of SICI (Roshan et al., 2003; Vucic et al., 2009). While axonal refractoriness might partially explain SICI at 1 ms, it cannot entirely explain this inhibition (Ni et al., 2007; Roshan et al., 2003) as

there is some evidence that synaptic inhibition plays a role in SICI at 1 ms as well as at longer ISIs (Ni et al., 2007; Roshan et al., 2003; Vucic et al., 2009). Here we provide evidence that a reduction in neither of these independent SICI processes can explain the increase in corticospinal excitability of proximal muscle representations during INB.

The current LICI results are important for two reasons. First, despite an increase in corticospinal excitability of the forearm flexors during INB at the wrist, there was no reduction in LICI acting on these muscle representations; indeed LICI measured with an ISI of 150 ms *increased* during INB. This finding shows that a reduction in LICI cannot explain the increase in corticospinal excitability observed in the forearm flexors. Second, the differential modulation of $LICI_{80}$ and $LICI_{150}$ provides strong evidence for distinct LICI processes. While there is strong evidence that distinct phases of SICI occur at different ISIs, at present there is only preliminary evidence for the existence of independent LICI processes with different time courses. Chu and colleagues showed that while LICI at 100 ms and LICI at 150 ms inhibited MEPs to a similar extent, LICI at 100 ms but not 150 ms suppressed SICI (Chu et al., 2008; Chu et al., 2009) leading these authors to suggest that LICI at an ISI of 100 ms and LICI at 150 ms are mediated by different mechanisms. As $GABA_B$ receptors, which are known to mediate LICI (McDonnell et al., 2006; Muller-Dahlhaus et al., 2008; Sanger et al., 2001; Werhahn et al., 1999), are found at both presynaptic and postsynaptic locations whereas $GABA_A$ receptors, known to mediate SICI (Di Lazzaro et al., 2000a; Hanajima et al., 1998), are primarily located on the postsynaptic membrane, Chu and colleagues (2008) suggested that LICI at 100 ms and LICI at 150 ms are mediated by pre- and postsynaptic $GABA_B$ receptor activity, respectively. It may be that INB does not affect presynaptic $GABA_A$ or $GABA_B$ receptor activity, shown by the absence of any change in SICI or $LICI_{80}$ during INB, but does increase postsynaptic $GABA_B$ receptor activity, shown by the increase

in $LICI_{150}$ during INB. The increase in postsynaptic $GABA_B$ activity resulting in greater $LICI_{150}$ during INB, however, cannot explain the increase in corticospinal excitability evident during INB. Indeed, this paradoxical finding suggests some other process (or processes) must mediate the increase in excitability in the presence of an increase in $LICI_{150}$, or that this other process (or processes) underlies both the corticospinal excitability increase and the increase in $LICI_{150}$ observed during INB.

It has been suggested that the down-regulation of GABAergic cortical circuits is important in deafferentation-induced plasticity (Jacobs and Donoghue, 1991; Sanes and Donoghue, 2000), and Levy and colleagues (2002) showed a decrease in GABA levels in M1 measured using magnetic resonance spectroscopy (MRS) during INB. (But note that while MRS provides information about the concentration of neurochemicals within a specified region of interest, it does not provide information about synaptic activity (Stagg et al., 2011). More direct evidence for a possible role of GABAergic circuits in INB-induced plasticity comes from Werhahn and colleagues (2002) who showed that Lorazepam, primarily a $GABA_A$ receptor agonist, suppressed but did not abolish the INB-induced increase in corticospinal excitability. $GABA_A$ circuits are widespread throughout the cortex and are also present in the spinal cord, where they are thought to be important in the control of somatosensory transmission (Sivilotti and Woolf, 1994). One possible explanation for Werhahn's finding is that during INB Lorazepam acted to up-regulate $GABA_A$ receptor activity both in the spinal cord and the cortex, thereby attenuating the loss of somatosensory input (which is probably important in INB-induced plasticity), and in turn diminishing changes at the cortex that would normally result from the substantial reduction in somatosensory input caused by the INB.

SICF in the forearm flexors decreased systematically with increasing ISI as expected (Chen and Garg, 2000; Hanajima et al., 1998; Tokimura et al., 1996), but did not change at any of the tested ISIs 15 minutes after cuff inflation despite a 46% increase in the amplitude of MEPs elicited from the forearm flexors. The first peak of SICF is thought to reflect the excitability of the initial axonal segments, excited subliminally by S1, while the later peaks are thought to reflect the summation of excitatory synaptic activity (Hanajima et al., 2002; Ortu et al., 2008). The current results suggest that neither a decrease in the threshold for exciting the initial axonal segments, nor an increase in the excitability of those excitatory interneuronal circuits probed by the SICF protocol, can explain INB-induced increases in corticospinal excitability.

While the neurophysiological origins of INB-induced plastic changes remain unknown, the present results allow us to rule out three plausible candidates. One possible alternate explanation is that INB-induced plasticity might be due to the release of inhibitory processes acting on M1 from other brain regions. Non-human primate studies show that the hand area in M1 receives somatosensory input (Darian-Smith and Darian-Smith, 1993; Friedman and Jones, 1981). In humans, peripheral nerve stimulation which precedes a single suprathreshold TMS pulse can suppress the amplitude of the MEP elicited by the TMS at both short (~20-40 ms) and long (~200-300 ms) ISIs (Tokimura et al., 2000). There is evidence that both short and long afferent inhibition are cortical as neither H-reflexes (Delwaide and Olivier, 1990; Tokimura et al., 2000) nor F-waves (Chen et al., 1999; Sailer et al., 2003) are suppressed following peripheral nerve stimulation. Evidence for the physiological processes mediating these afferent inhibitory processes is sparse; one pharmacological study suggests a role for both GABAergic and cholinergic systems in short-latency afferent inhibition (Di Lazzaro et al., 2000b). While there is evidence of interactions between afferent inhibition and

intracortical inhibition (Aimonetti and Nielsen, 2001; Alle et al., 2009; Sailer et al., 2002; Sailer et al., 2003; Stefan et al., 2002; Udupa et al., 2009), it is thought that distinct inhibitory interneurons mediate, at least in part, these different types of inhibition (Alle et al., 2009; Udupa et al., 2009). Thus, it is possible that the loss of sensory input caused by INB could lead to the release of afferent inhibition which might in turn produce the increase in corticospinal excitability observed after INB.

There is increasing interest in exploiting the plastic capacity of the human motor cortex to develop targeted rehabilitation therapies (for reviews see Dobkin, 2008; Fox, 2009), but in order to develop effective therapies it is essential to identify the processes and mechanisms underlying such plasticity. In the current study we have shown that the modulation of SICI, LICI, or SICF alone cannot explain the rapid plastic changes induced by INB. Future studies should investigate 1) whether the INB-induced increase in corticospinal excitability can be partially accounted for by changes at the spinal level, and 2) which processes underlie INB-induced changes within the cortex.

Acknowledgements

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Fig. 1. Mean recruitment curves showing $SICI_1$ (left) and $SICI_2$ (right) as a function of S1 intensity at baseline (open symbols) and 30 minutes following inflation of the cuff (closed symbols). SICI is expressed as a ratio of paired-pulse to single-pulse MEP amplitude (larger ratios indicate less inhibition). Error bars show +/- SEM.

Fig. 2. Mean recruitment curves showing $LICI_{80}$ (left) and $LICI_{150}$ (right) as a function of S1 intensity at baseline (open symbols) and 30 minutes following inflation of the cuff (closed symbols). LICI is expressed as a ratio of paired-pulse to single-pulse MEP amplitude (larger ratios indicate less inhibition). Error bars show +/- SEM.

Fig. 3. Means of each subject's mean MEP amplitude ratios for each ISI at baseline (open symbols) and 15 minutes following cuff inflation (closed symbols). SICF expressed as a ratio of paired-pulse to single-pulse MEP amplitude (larger ratios indicate more facilitation). Error bars show +/- SEM.
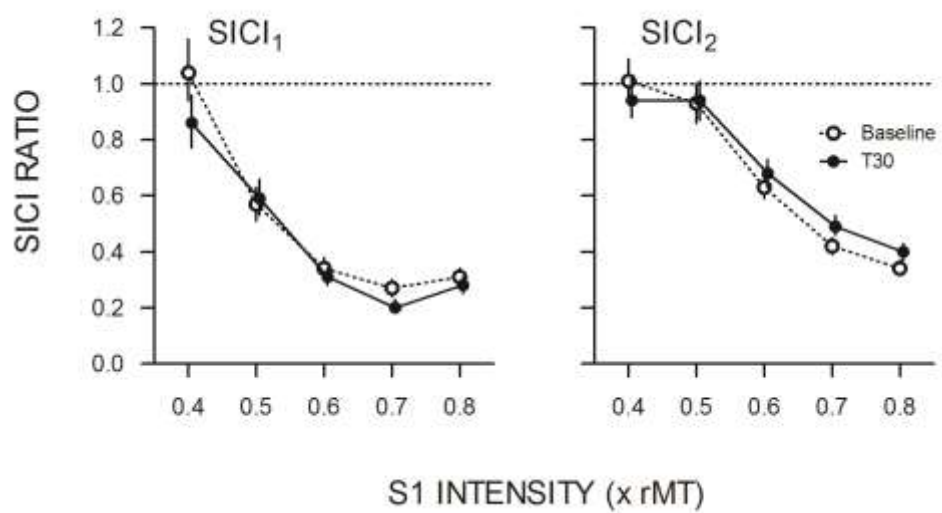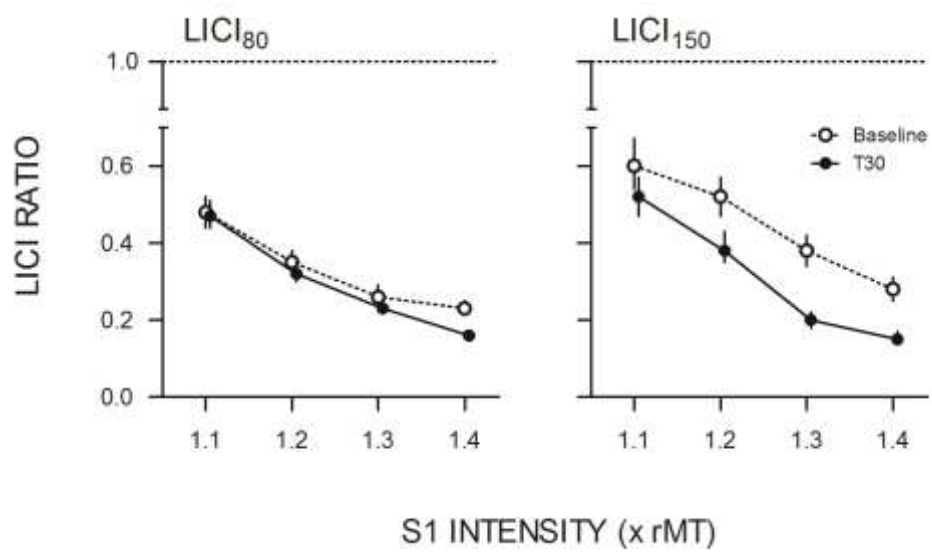
Fig. 1.

Fig. 2.

S1 INTENSITY (x rMT)

Fig. 3.