Organization of Ipsilateral Excitatory and Inhibitory Pathways in the Human Motor Cortex

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Abstract

Motor cortex stimulation has both excitatory and inhibitory effects on ipsilateral muscles. Excitatory effects can be assessed by ipsilateral motor-evoked potentials (iMEPs). Inhibitory effects include an interruption of ipsilateral voluntary muscle activity known as the silent period (iSP), and a reduction in corticospinal excitability evoked by conditioning stimulation of the contralateral motor cortex (interhemispheric inhibition, IHI). Both iSP and IHI may be mediated by transcallosal pathways. Whether iSP and IHI represent the same phenomenon and their relationship to the contralateral corticospinal projection remain unclear. The neuronal population activated by transcranial magnetic stimulation (TMS) is highly dependent on the direction of the induced current in the brain. We examined the relationship between iMEP, iSP, IHI and the contralateral corticospinal system by examining the effects of different stimulus intensities and current directions. Surface EMG was recorded from both first dorsal interosseous (FDI) muscles. The iSP in the right FDI muscle was obtained by right motor cortex stimulation during voluntary muscle contraction. IHI was examined by conditioning stimulation of the right motor cortex followed by test stimulation of the left motor cortex at interstimulus intervals (ISIs) of 2 to 80 ms. The induced current directions tested in the right motor cortex were anterior medial (AM), posterior medial (PM), posterior lateral (PM) and anterior lateral (AL). Contralateral MEPs (cMEPs) had the lowest threshold with the AM direction and the shortest latency with the PM direction. iMEPs were present in 8 out of 10 subjects. Both iMEP and IHI did not show significant directional preference. iSP was observed in all subjects with the highest threshold for the AL direction and the longest duration for the AM direction. cMEP, iSP and IHI all increased with stimulus intensity up to about 75% stimulator output. Target muscle activation decreased IHI at 8 ms ISI but had little effect on IHI at 40 ms ISI. iSP and IHI at 8 ms ISI did
not correlate at any stimulus intensities and current directions tested, and factor analysis showed that they are explained by different factors. However, active IHI at 40 ms ISI was explained by the same factor as iSP. The different directional preference for cMEP compared to iMEP and IHI suggest that these ipsilateral effects are mediated by populations of cortical neurons that are different from those activating the corticospinal neurons. iSP and IHI do not represent the same phenomenon and should be considered complementary measures of ipsilateral inhibition.
Introduction

Transcranial magnetic stimulation (TMS) of the motor cortex produces both excitatory and inhibitory effects on ipsilateral muscles. The ipsilateral excitatory effect can be measured by ipsilateral motor evoked potentials (iMEPs) (Wassermann et al. 1991; Ziemann et al. 1999). Because large iMEPs were obtained in a patient with complete agenesis of the corpus callosum (Ziemann et al. 1999), it was suggested that direct descending oligosynaptic pathways from the ipsilateral motor cortex are likely more important than interhemispheric mechanisms in mediating ipsilateral excitatory responses. However, iMEPs in this patient may not be mediated by the same pathways as in normal subjects, and the latency differences between iMEPs and cMEPs can be accounted for by transcallosal facilitation (Hanajima et al. 2001) of the contralateral motor cortex.

Ipsilateral inhibitory effects induced by motor cortex stimulation includes an interruption of ongoing voluntary EMG activity, known as the ipsilateral silent period (iSP) (Ferbert et al. 1992; Meyer et al. 1995). iSP has been proposed as a simple clinical diagnostic tool for callosal function (Meyer et al. 1999), and was found to be delayed or prolonged in neurological disorders such as multiple sclerosis (Schmierer et al. 2000) and writer’s cramp (Niehaus et al. 2001). Another ipsilateral inhibitory phenomenon is interhemispheric inhibition (IHI) that can be elicited by conditioning stimulation of the ipsilateral motor cortex followed by test stimulation of the contralateral motor cortex. At interstimulus intervals (ISIs) between 6 and 50 ms, the conditioning TMS reduced the contralateral MEP (cMEP) generated by the test stimulus (Ferbert et al. 1992; Gerloff et al. 1998). IHI was more prominent following conditioning stimulation of the dominant compared to the non-dominant hemisphere (Netz et al. 1995) and was found to be
reduced in musicians (Ridding et al. 2000) and in patients with schizophrenia (Daskalakis et al. 2002a).

The pathways mediating ipsilateral responses and their relationship to the contralateral corticospinal projection have not been fully defined. One possibility involves interhemispheric connections such as the corpus callosum projecting to the contralateral motor cortex. Another hypothesized mechanism is direct descending oligosynaptic pathways from the ipsilateral cortex. This may be mediated by pathways that are in part bilaterally organized, such as the reticulospinal tract (Nathan et al. 1996), spinal interneuronal circuits (Gracies et al. 1994; Mazevet et al. 1996; Roby-Brami and Bussel 1987; Shahani and Young 1971), the uncrossed corticospinal tract (Yakolev and Rakic 1966) and the propriospinal pathway (Ziemann et al. 1999). Both mechanisms may be involved and the structural or functional redundancy of normal human motor control may become crucial in case of lesions (Gerloff et al., 1998). Although previous studies (Ferbert et al. 1992) have suggested that iSP and IHI represent the same phenomena, this has not been formally investigated.

The population of cortical neurons activated by TMS is dependent on the direction of the induced current. Medially oriented induced current produced shorter latency MEPs in contralateral hand muscles compared to anterior or posterior directed current (Sakai et al. 1997; Werhahn et al. 1994; Wilson et al. 1996). Epidural recording of corticospinal waves confirmed that medially directed current may activate corticospinal axons directly leading to direct (D) waves whereas anteriorly directed current activate corticospinal neurons indirectly leading to indirect (I) waves (Di Lazzaro et al. 1998; Kaneko et al. 1996). Based on the latency of EMG responses, Sakai et al. (1997) hypothesized that different directions of induced current in the brain preferentially activate different I-waves. Direct recording of corticospinal volleys also
found that anterior and posterior directed currents elicited corticospinal volleys of different peak latencies and duration suggesting that they activate different cortical circuits (Di Lazzaro et al. 2001). Since the site of excitation by TMS is probably at the initial bend of the axon where the induced current led to an outward membrane current (Amassian et al. 1992), these findings are likely related to different orientations of various groups of cortical fibers.

In the present study, we examined the relationship between iMEP, iSP, IHI and the contralateral corticospinal system by examining the effects of different stimulus intensities and current directions. We hypothesize that if these phenomena are mediated by different populations of cortical neurons, the effects of current direction and stimulus intensity may differ.

**Methods**

**Subjects**

We studied 19 right-handed normal volunteers (14 men and 5 women, mean age 47.8 years, range 21 to 72 years). Handedness was assessed by the Edinburgh inventory (Oldfield 1971). All subjects gave written informed consent and the protocol was approved by the University Health Network Research Ethics Board.

**EMG recording**

Surface EMG was recorded from the first dorsal interosseous (FDI) muscles bilaterally with disposable disc electrodes in a tendon-belly arrangement. EMG was monitored on a computer screen and via speakers at high gain. In the experiments that required the subject to maintain a constant contraction, the EMG passed through a leaky integrator and the EMG level was displayed on an oscilloscope to allow the subject maintained a constant contraction with auditory and visual feedback. The signal was amplified (Intronix Technologies Corporation
Model 2024F, Bolton, Ontario, Canada), filtered (band pass 2 Hz to 5 kHz), digitized at 5 KHz (Micro 1401, Cambridge Electronics Design, Cambridge, U.K.) and stored in a laboratory computer for offline analysis (Signal 1.80 software).

**Ipsilateral MEP and iSP**

TMS was performed using Magstim 200 stimulators (The Magstim Company, Dyfed, United Kingdom) and figure-of-eight coils (mean diameter 70mm, max strength 2.2 T). TMS was applied to the right motor cortex and four directions of induced current in the brain were studied: Anterior Medial (AM), Posterior Medial (PM), Posterior Lateral (PL) and Anterior Lateral (AL) (Fig. 1). For each current direction, the optimal coil position, rest and active motor thresholds (MT) for activating the contralateral (left) FDI muscle was first determined. Rest MT was the minimum stimulator output that produced MEPs of \( \geq 50 \text{ V} \) in at least 5 out of 10 trials. Active MT was the minimum stimulator output that produced MEPs of \( \geq 100 \text{ V} \) in at least 5 out of 10 trials with a constant background contraction of 20% of the maximum integrated EMG.

The four coil orientations were studied in separate runs in random order. The subjects maintained a 50% maximum contraction of the right FDI muscle with visual and auditory feedback during testing for iMEP and iSP. Breaks within each experimental run are allowed to avoid fatigue. The first set of experiments involved 10 subjects. The right motor cortex was stimulated at 5 different intensities at 1.5, 1.75, 2 and 2.25 times the active MT (for left FDI muscle) and at 100% of the stimulator output. Thresholds for iMEP and iSP were calculated from this set of experiments. The second set of experiments involved 9 subjects. We tested four stimulus intensities at 45, 60, 75 and 90% of the stimulator output. The different stimulus intensities were presented in random order. Each stimulus intensity was repeated 10 times and the stimuli were presented 4.5 seconds apart. We also examined the contralateral MEP (cMEP)
with 10 TMS pulses to the right motor cortex at 1.25 times the active MT while the subject maintaining a 50% maximum contraction of the contralateral left FDI.

*Interhemispheric Inhibition at rest*

The protocol used was similar to that described by Ferbert et al. (Ferbert et al. 1992). The subjects relaxed both FDI muscles during the experiment. The conditioning stimuli were applied to the right motor cortex at four current directions (AM, PM, PL, AL) in random order in separate runs. The test stimuli were applied to the left motor cortex in the AM direction and were adjusted to evoke about 1 mV MEPs in the right FDI muscle. In the first set of experiments (10 subjects), the conditioning stimulus (to right motor cortex) was adjusted to evoke MEPs of about 1.5 mV in the left FDI muscle. ISIs of 2, 5, 6, 8, 10, 20, 50 and 80 ms were tested. Each run consisted of 10 trials of the test pulse alone and 10 trials of each ISI delivered in random order (90 trials). In the second set of experiments (9 subjects), we examined the effects of different intensities of the conditioning pulse. The conditioning pulse (to the right motor cortex) at 45, 60, 75 and 90% of the stimulator output were tested in separate runs. ISIs of 8 ms and 40 ms were used. Each run consisted of 10 trials of the test pulse alone and 10 trials of each ISI delivered in random order (30 trials).

*Interhemispheric inhibition during muscle activation*

In this experiment we examined IHI and iSP during voluntary contraction. Ten subjects participated. The subjects maintained a 50% maximum contraction of the right FDI muscle with visual and auditory feedback. The conditioning stimuli were applied to the right motor cortex at four current directions (AM, PM, PL, AL) in random order at 45, 60, 75 and 90% of the stimulator output in separate runs. The test stimuli were applied to the left motor cortex in the AM direction and were adjusted to evoke MEPs of about 1.5 mV in the right FDI muscle during
50% maximum voluntary contraction. ISIs of 8 ms and 40 ms were tested. Each run consisted of 10 trials of the test pulse alone, 10 trials of the conditioning pulse alone and 10 trials of each ISI delivered in random order (40 trials). The trials with the conditioning pulse alone were used to measure iSP and iMEP.

Data analysis

The iMEP and iSP data were analyzed in Signal 1.80 software using customized scripts. Since iMEP and iSP were generally of small amplitude and were variable from trial to trial, we used automated statistical methods to define their presence. The criteria chosen were based on our preliminary studies to distinguish the changes from background noise. An example of the measurements is shown in Fig. 2. For each subject, the surface EMG from the right FDI muscle for each stimulus intensity and coil orientation were rectified and averaged. The mean and standard deviation of the baseline EMG level for 100 ms before TMS was determined. An iMEP was deemed to be present if the poststimulus EMG exceeded the prestimulus mean by more than 1 standard deviation for at least 5 ms (25 consecutive data points based on 5 KHz sampling rate). iMEP onset was defined as last crossing of the mean baseline EMG level before the iMEP peak and iMEP offset the first crossing of the mean baseline EMG level after the iMEP peak. iMEP area was calculated between the iMEP onset and offset. Similarly, iSP was deemed significant if the poststimulus EMG fell below the prestimulus mean by at least 1 standard deviation for more than 5 ms (25 consecutive data points based on 5 KHz sampling rate). The iSP onset, offset, duration and area were calculated similar to that for the iMEP. In some subjects the iSP was interrupted by “rebound” potential (Fig. 2) and the iSP onset and offset boundaries included all significant iSP areas. The iSP duration was the time between the onset and offset values. iMEP
and iSP thresholds were the lowest stimulus intensities for which we found a significant response.

For IHI, the peak-to-peak MEP amplitude for each trial for the right FDI muscle was analyzed offline. The inhibition or facilitation for each trial was expressed as a ratio of the mean conditioned to unconditioned MEP amplitude for each subject. Ratios less than one indicate inhibition, and ratios greater than one indicate facilitation.

Statistical Analysis

For iMEP (thresholds, amplitude, latency), cMEP (thresholds, amplitude, latency) and iSP (threshold, duration and areas), the effects of stimulus intensities and current direction were examined by analysis of variance (ANOVA) in a factorial design. Similarly, for IHI the effects of ISI, conditioning stimulus intensities and current direction were examined by ANOVA. If ANOVA showed a significant effect, post-hoc testing (Fisher’s PLSD) was used to examine the differences among different current directions and stimulus intensities.

The relationship between IHI and iSP was explored using simple correlation and factor analysis. Factor analysis is a method to examine the relationship between correlated variables. Principal component factor analysis with Varimax rotation was used to evaluate the relationship between conditioning stimulus intensity, cMEP amplitude, IHI at 8 ms, IHI at 40 ms, iSP area and iSP duration. Separate analyses were performed for rest and active IHI.

Statview 5.01 software (SAS Institute Inc., Cary, NC) was used for statistical analysis. Differences were considered significant if p<0.05 except for correlation between IHI and iSP where p<0.01 was considered significant because multiple comparisons were performed. Unless otherwise stated, values are expressed as mean ± standard deviation.
Results

$iMEP$ and $cMEP$

An example of $iMEP$ and $iSP$ is shown in Fig. 2. In the 10 subjects tested in the first set of experiment (stimulus intensities from 1.25 to 2.25 times the active $cMEP$ threshold and at maximum stimulator output), $iMEP$ was detected in four subjects in the AM direction, four subjects in PM direction, five subjects in the PL direction and two subjects in the AL direction. When all current directions were considered, $iMEP$ was present in eight out of ten subjects. The thresholds are much higher for $iMEP$ than $cMEP$ (Fig. 3A). The effect of current direction on active $cMEP$ threshold was significant (ANOVA, $p<0.001$) and post-hoc testing showed that the threshold for AM direction was significantly lower than all the other directions ($p <0.001$) whereas the other three directions were not significantly different. By contrast, there was no significant effect of current direction for $iMEP$.

Combining the data from the first and second series of experiments, the amplitude for $cMEP$ ($2.22 \pm 1.90$ mV) for the relaxed left FDI muscle was much higher than that for $iMEP$ ($0.48 \pm 0.30$ mV) simultaneously recorded from the active right FDI muscle. There was no significant effect of current direction on $cMEP$ amplitude in the first series of experiments, where the stimulus intensities used were a percentage of the active $cMEP$ motor threshold for each direction. In the second series of experiment with fixed stimulus intensity, the effect of current direction on $cMEP$ amplitude was significant and post-hoc testing showed that AM direction resulted in higher $cMEP$ amplitude than the other three directions. The effect of current direction was not significant for $iMEP$ amplitude for either series of experiment.

The $iMEP$ latency ($26.1 \pm 4.2$ ms) from the active right FDI muscle was significantly longer than the $cMEP$ latency from the relaxed left FDI muscle ($21.2 \pm 4.0$ ms, $p<0.0001$, paired-
t test)(Fig. 3B). This difference in latency was influenced by two factors: the cMEP amplitudes were much larger than that of iMEP which increased the difference and cMEPs were measured in relaxed muscle while iMEPs were measured in active muscle which decreased the difference. The ANOVA showed a significant effect of current direction on cMEP latency (p<0.0001) and post-hoc testing showed that the cMEP latency for PM direction was shorter than the other three directions. Current direction had no significant effect on iMEP latency.

Ipsilateral Silent Period

The first series of experiments were used to examine iSP threshold and the results are shown in Fig. 4. iSP was detected in all subjects tested. With iSP threshold expressed as a ratio of the active MT, ANOVA showed a significant effect of current direction (p=0.018). Post-hoc testing demonstrated that the iSP threshold for AM direction was significantly higher than that for PM (p=0.009) and PL (p=0.037) directions. When the iSP thresholds were converted to percentages of the stimulator output, ANOVA also showed significant effect of current direction (p=0.029). Post-hoc testing showed that the AL direction had significantly higher iSP threshold than the AM (p=0.007) and PM (p=0.0113) directions.

We used the second series of experiments to examine the effects of stimulus intensity on iSP duration and iSP area. ANOVA showed that the effects of both stimulus intensity (p<0.001) and current direction (p=0.025) on iSP duration were significant but their interaction was not (Fig. 5A). For iSP area the effect of stimulus intensity was significant but effect of current direction was not (Fig. 5B). Post-hoc testing showed that both iSP area and duration increased with stimulus intensity up to 75%, whereas the values for 75% and 90% were similar. The AM direction had longer iSP duration than the PM direction (p=0.0025), whereas the other directions
were not significantly different from each other. The iSP latency was 36.9 ± 7.7 ms and was not affected by stimulus intensity or current direction.

*Interhemispheric inhibition at rest*

The results from the first series of experiments are shown in Fig. 6. IHI was evident from 8 to 50 ms for all current directions. The effect of ISI (p<0.0001) on IHI was significant but the effect of current direction was not. Using the result at ISI of 8 ms, the cMEP amplitude in the left FDI muscle evoked by conditioning stimulus did not correlate with the strength of IHI ($r^2=0.027$).

The effects of conditioning stimulus intensity on IHI at ISI of 8 ms is shown in Fig. 7A and IHI at ISI of 40 ms is shown in Fig. 7B. The effects of stimulus intensity were significant (p<0.0001) for both ISIs of 8 ms and 40 ms but the effects of current direction were not. Similar to the iSP area and duration, the IHI for 8 and 40 ms ISIs both increased with the stimulus intensity from 45% to 75% of stimulator output. There was no significant difference in IHI between 75% and 90% of stimulator output (Fig. 7A & B). The cMEP amplitude evoked by the conditioning stimulus in the left FDI muscle is shown in Fig. 7E. The effects of both stimulus intensity (p<0.0001) and current direction (p=0.0001) on cMEP were significant but their interaction was not. As expected, post-hoc testing showed that the cMEP amplitude is higher in the AM direction compared to the other three directions.

*Interhemispheric inhibition during muscle activation*

The results for ISI of 8 ms are shown in Fig.7C and that for ISI of 40 ms are shown in Fig. 7D. At ISI of 8 ms, there was much less IHI in the active (Fig. 7C) compared to the rest condition (Fig. 7A). By contrast, at ISI of 40 ms IHI in the active (Fig. 7D) and rest (Fig. 7B) conditions were comparable. Similar to IHI at rest, the effects of stimulus intensity were
significant for both ISIs of 8 ms (p=0.003) and 40 ms (p<0.0001) but the effects of current
direction were not.

We also examined whether iMEPs may contribute to reduced IHI at ISIs of 8 ms during
muscle activation. The presence of iMEPs was determined from the trials with the conditioning
pulse alone. iMEPs were found in 7 runs (amplitude 0.27 ? 0.36 mV) in 5 subjects out of 160
experimental runs (10 subjects x 4 stimulus intensities x 4 current directions) performed. In
these 7 runs the test MEP ratio for ISI of 8 ms was 0.94 ? 0.11, very similar to the data for the
entire group shown in Fig. 7C.

**Relationship between IHI and iSP**

Rest IHI at ISI of 8 ms did not show significant correlation with iSP duration or iSP area
at each of the stimulus intensities (45, 60, 75 and 90% of stimulator output) and current
directions (AM, PM, PL, AL) examined. When the stimulus intensities were combined, IHI
significantly correlated with iSP duration (p value ranged from 0.0156 to < 0.0001) and iSP area
(p value ranged from 0.0155 to < 0.0001) for all current directions. This is probably because
both IHI and iSP increased with stimulus intensity. Rest IHI at 40 ms ISI did not correlate with
iSP area for any of the combination of stimulus intensities and current directions examined but it
correlated with iSP duration for AM direction at 60% (p=0.003, r=0.86) and 75% (p=0.01,
r=0.55) stimulator output.

Active IHI at ISI of 8 ms did not show significant correlation with iSP duration or iSP
area at each of the stimulus intensities and current directions examined. Active IHI at ISI of 40
ms did not correlate with iSP area but correlated with iSP duration at 75% stimulator output for
AM (p=0.004, r=0.81) and AL (p=0.003, r=0.82) current directions.
Factor analysis was performed to examine the relationship between stimulus intensity, cMEP amplitude, IHI at ISI 8 ms, IHI at ISI of 40 ms, iSP area and ISP duration. The values for the oblique solution reference structures for rest IHI is shown in Table 1A while the values for active IHI are shown in Table 1B. For rest IHI, the Eigenvalues for the first three components were 3.79, 0.70 and 0.54. The three factor solution explained 83.8% of the variance. None of three factors showed strong association between rest IHI at 8 ms and iSP area or iSP duration, although Factor 2 showed a weak associated between rest IHI at 40 ms and iSP area and duration. For active IHI, the Eigenvalues for the first three components were 3.67, 0.84 and 0.58. The three factor solution explained 84.8% of the variance. Factor 1 showed a strong association between active IHI at 40 ms and iSP area and duration and Factor 2 showed a weak relationship between active IHI at 8 ms and iSP area.

Discussion

Ipsilateral motor-evoked potentials

We found iMEPs in most subjects in at least one current direction with high stimulus intensities and background muscle activation, although with any given current direction iMEPs were observed in only a minority of subjects. iMEPs are also of higher motor threshold, much smaller amplitude and longer latencies compared to cMEPs. Eliciting iMEP in hand muscles may require testing of multiple current directions. These findings confirm that circuitry for ipsilateral MEPs is present in most subjects but is sparse compared to the contralateral corticospinal projections. Previous studies of iMEP for the FDI muscle (Wassermann et al. 1994; Ziemann et al. 1999) had similar findings.
Similar to previous studies (Sakai et al. 1997; Werhahn et al. 1994), we found that cMEPs have the lowest threshold for the AM direction and shortest latency for the PM direction (Fig. 3). Medially directed current may stimulate corticospinal neurons directly whereas anteriorly directed current predominately activate corticospinal neurons transsynaptically (Kaneko et al. 1996; Werhahn et al. 1994). By contrast, we found no preferred current direction for iMEP thresholds (Ziemann et al. 1999) and iMEP latencies. Since the directional preference and latencies for iMEPs were markedly different from cMEPs, iMEPs are probably mediated by cortical neurons that different from those mediating the cMEPs and likely involve pathways separate from the fast corticospinal tract. This is consistent with mapping studies which showed that optimal scalp positions for eliciting iMEPs are more lateral than that for cMEPs (Wassermann et al. 1994; Ziemann et al. 1999). If this argument is correct, iMEPs are probably not related to transcallosal facilitation because transcallosal facilitation may be mediated by collaterals of corticospinal neurons activated by the conditioning stimulus (Hanajima et al. 2001). However, the effects of current direction on iMEPs should be interpreted with caution because iMEPs are absent in some subjects and their high variability probably due to conduction along a polysynaptic pathway may obscure any effect of current direction in the cortex.

Ipsilateral silent period

Since patients with agenesis or surgical lesions of the corpus callosum had absent or delayed iSP, it is likely that the iSP is at least in part mediated by fibers passing through the corpus callosum (Meyer et al. 1995; Meyer et al. 1998). This is consistent with preservation of iSP in patients with subcortical cerebrovascular lesions that interrupted the corticospinal tract but not the corpus callosum (Boroojerdi et al. 1996). Furthermore, in pre-school children who have yet to develop a functionally competent corpus callosum, there was no detectable iSP (Heinen et
Nevertheless, transcallosal connection between cortical hand motor representation in primates is sparse (Gould, III et al. 1986; Rouiller et al. 1994).

Similar to previous reports (Ferbert et al. 1992; Wassermann et al. 1991), iSP was easily elicited in all subjects tested and iSP thresholds were much lower than iMEP thresholds (Fig. 3A & 4). iSP is influenced by current direction. The higher iSP threshold expressed as a ratio to the active cMEP threshold for the AM direction (Fig. 4) can be explained by its lower cMEP threshold compared to the other current directions (Fig 3A). With iSP threshold expressed as an absolute stimulator output, AL direction has the highest iSP threshold (Fig. 4). By contrast, although AM and PM directions have similar iSP thresholds, the stimulus-response curve was steeper for the AM direction (Fig. 5). The longer iSP in the AM direction is similar to the results of a previous abstract (Meyer et al. 1996).

A previous study (Meyer et al. 1995) that used anterior-posterior current (similar to our AM direction) found that iSP duration increased rapidly from 50% to 60% stimulator output followed by a smaller increase up to 80% stimulator output. This is similar to our findings for the AM direction (Fig. 5) which showed that iSP increased with higher stimulus intensities up to 75% stimulator output with no significant difference 75 and 90%. It was also reported that iSP latency decreased with increasing intensity and reached a minimum at 80% stimulator output (Meyer et al. 1995) whereas we found no change in iSP latency with stimulus intensity. This difference is probably related to how iSP latency was measured. Meyer et al. (Meyer et al. 1995; Meyer et al. 1998) used a visual method to measure iSP and defined iSP latency as the point where the averaged EMG activity clearly fell under the mean prestimulus EMG level. Because the depth of iSP increases with stimulus intensity, it can be expected that iSP latency will decrease with stimulus intensity with this method of analysis. We used an automated method
and iSP onset was defined as the last crossing of the mean prestimulus EMG level before the iSP. With this method, the onset latency should not depend on the depth of iSP. This interpretation is consistent with the finding that our mean iSP latency of 36.9 ms is similar to the minimum iSP latency of 36 to 37 ms reported by Meyer et al. (Meyer et al. 1995).

Interhemispheric Inhibition

IHI at short ISIs (6 to 12 ms) is at least in part due to inhibition at the cortical level since the conditioning stimulus had no effect on test responses evoked by a small anodal electric stimulus and did not change spinal excitability as measured by H-reflex in the ipsilateral muscles (Ferbert et al. 1992). Direct measurement of corticospinal waves also demonstrated that TMS of the ipsilateral motor cortex reduced the excitability of the contralateral motor cortex (Di Lazzaro et al. 1999). IHI was absent in a single subject with agenesis of the corpus callosum (Rothwell et al. 1991). However, subcortical mechanisms may also be involved since TMS reduced the response in ipsilateral muscles to electrical stimulation of the pyramidal tract at the level of the pyramidal decussation (Gerloff et al. 1998).

The inhibition of test MEPs between ISIs of 8 and 50 ms is similar to previous reports (Ferbert et al. 1992; Gerloff et al. 1998). Although transcallosal facilitation has been reported at ISIs of 4 to 5 ms, we did not observe any facilitation at 5 ms. This is probably because we used induced current in the AM direction to elicit the test MEPs whereas transcallosal facilitation was observed only if the test MEPs were produced by posteriorly directed current at low stimulus intensities (Hanajima et al. 2001).

The neurons responsible for IHI are probably not directionally specific and are separate from the contralateral corticospinal system mediating cMEPs. This is because the strength of
IHI did not correlate with cMEP amplitude evoked by the conditioning stimulus and IHI has no significant directional preference whereas cMEP showed strong directional preference.

Most of the previous studies on IHI concentrated on short ISIs from about 6 to 12 ms (Daskalakis et al. 2002b; Di Lazzaro et al. 1999; Ferbert et al. 1992; Netz et al. 1995; Ridding et al. 2000) and ISIs longer than 20 ms have not been systematically investigated. Our findings suggested that there are significant differences between IHI at short (~8 ms) and long (~40 ms) ISIs. Similar to the results of Ridding et al. (Ridding et al. 2000), we found that IHI at 8 ms was reduced with target muscle activation (Fig. 7A and C). This is unlikely to be due to iMEPs because iMEPs are rare, are of much smaller amplitudes than cMEPs and in the experimental runs where we found iMEPs the degree of inhibition during muscle activation was very similar to that of the entire group. By contrast, IHI at 40 ms showed little change with muscle activation (Fig. 7B and D). Moreover, factor analysis suggested that IHI at 40 ms is related to iSP whereas IHI at 8 ms is not. Therefore, IHI at 8 ms and 40 ms are not mediated by the same mechanism.

Relationship between iSP and IHI

Although both iSP and IHI represent ipsilateral inhibition, whether the same mechanism mediates iSP and IHI has not been previously studied. This is an important issue since abnormalities of iSP and IHI at ISI of 6 to 12 ms have been found in several neurological and psychiatric disorders and these techniques may have diagnostic applications. One difference between iSP and IHI is that iSP represent interruption of voluntary muscle activity while IHI reflects inhibition of synchronized activation of the corticospinal system induced by the test stimulus. However, iSP and IHI are similar in several ways. It has been suggested both iSP (Meyer et al. 1995) and IHI (Di Lazzaro et al. 1999; Ferbert et al. 1992) represent transcallosal inhibition. The responses of iSP and IHI to changes in stimulus intensities are similar.
Regardless of the current direction, both iSP and IHI increased with the intensity of the conditioning stimulus up to about 75% of stimulator output whereas the level of inhibition at 75% and 90% were not significantly different. Previous mapping studies found that the optimal scalp location eliciting both iSP (Meyer and Roericht 1996; Wassermann et al. 1994) and IHI (Ferbert et al. 1992) are the same as the optimal location for cMEPs. In patients with schizophrenia both iSP (Boroojerdi et al. 1999) and IHI at ISI of 10 ms (Daskalakis et al. 2002a) were abnormal.

However, several of our observations suggest that iSP and IHI are not mediated by the same mechanism. iSP showed directional preference whereas IHI did not. Although IHI measures only the depth of inhibition while iSP area measures both the depth and duration of inhibition, if these measures are mediated by the same mechanism they can be expect to correlate and be explained by the same factor in a factor analysis. For IHI at 8 ms, we found no correlation between iSP and IHI at any of the intensities and current directions tested. Factor analysis revealed that iSP and rest IHI at 8 ms are explained by different factors (Table 1A) and active IHI at 8 ms showed only a weak relationship with iSP area (Table 1B). On the other hand, IHI at 40 ms both at rest and during muscle activation significantly correlated with iSP duration for some of the stimulus intensities and current directions tested. Moreover, factor analysis showed that active IHI at 40 ms was strongly related to both iSP area and duration (Table 1B).

These observations suggest that similar circuits may mediate IHI at 40 ms and iSP. While our findings do not rule out an overlap between iSP and IHI at 8 ms and both may be related to transcallosal inhibition, they suggest that iSP and IHI at 8 ms do not represent the same phenomenon. Different neuronal population in the ipsilateral motor cortex may mediate iSP and IHI, perhaps though different sets of callosal fibers. Alternatively, different sets of target neurons
in the contralateral motor cortex may be responsible for the two types of inhibition. There is also

evidence that IHI may in part be mediated by subcortical circuits (Gerloff et al. 1998). Whatever

the mechanism, iSP and IHI should be considered complementary rather than equivalent

measures of ipsilateral inhibition.
Acknowledgments

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Table 1A: Factor Analysis - Oblique solution reference structure for rest IHI

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(63.2%)</td>
<td>(11.7%)</td>
<td>(8.9%)</td>
</tr>
<tr>
<td>Stimulus Intensity</td>
<td>0.580</td>
<td>0.058</td>
<td>-0.010</td>
</tr>
<tr>
<td>cMEP amplitude</td>
<td>0.676</td>
<td>-3.8x10^{-4}</td>
<td>-0.056</td>
</tr>
<tr>
<td>IHI 8 ms</td>
<td>0.074</td>
<td>0.058</td>
<td>0.717</td>
</tr>
<tr>
<td>IHI 40 ms</td>
<td>-0.060</td>
<td>-0.173</td>
<td>0.471</td>
</tr>
<tr>
<td>iSP duration</td>
<td>0.255</td>
<td>0.560</td>
<td>2.2x10^{-4}</td>
</tr>
<tr>
<td>iSP area</td>
<td>-0.050</td>
<td>0.811</td>
<td>-0.001</td>
</tr>
</tbody>
</table>

The numbers below each factor is the percentage of the variance in the data explained by each factor. The numbers in the table represent the correlation of each variable to each factor. Factor 1 is primarily related to stimulus intensity, cMEP amplitude and iSP duration. Factor 2 is related to iSP area, iSP duration and to a less extent IHI at 40 ms. Factor 3 is related to IHI at 8 ms and 40 ms. None of the factors are related to both IHI at 8 ms and iSP.
Table 1B: Factor Analysis - Oblique solution reference structure for active IHI

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulus Intensity</td>
<td>0.004</td>
<td>2.8x10^-5</td>
<td>0.852</td>
</tr>
<tr>
<td>cMEP amplitude</td>
<td>0.500</td>
<td>-0.072</td>
<td>0.271</td>
</tr>
<tr>
<td>IHI 8 ms</td>
<td>0.024</td>
<td>0.908</td>
<td>-0.011</td>
</tr>
<tr>
<td>IHI 40 ms</td>
<td>-0.678</td>
<td>-0.016</td>
<td>-0.038</td>
</tr>
<tr>
<td>iSP duration</td>
<td>0.697</td>
<td>0.064</td>
<td>0.143</td>
</tr>
<tr>
<td>iSP area</td>
<td>0.665</td>
<td>-0.264</td>
<td>-0.109</td>
</tr>
</tbody>
</table>

The numbers below each factor is the percentage of the variance in the data explained by each factor. The numbers in the table represent the correlation of each variable to each factor. Factor 1 is related iSP area, iSP duration, IHI at 40 ms and cMEP amplitude. Factor 2 is related IHI at 8 ms and to a less extent iSP area. Factor 3 is related to stimulus intensity and cMEP amplitude.
Figure Legends

Figure 1
A schematic representation of the current directions used for right motor cortex stimulation. The arrows represent the direction of the induced current. AM = Anterior Medial, PM = Posterior Medial, AL = Anterior Lateral and PL = Posterior Lateral.

Figure 2
Example of an iMEP followed by an iSP. Recording from right FDI muscle with the subject maintaining a 50% maximum voluntary contraction. Rectified and averaged EMG from 10 trials. The right motor cortex was stimulated at 100 ms (stimulus artifact) at 75% of stimulator output in the AM direction. An iMEP (onset 128.1 ms, peak amplitude 0.532 mV, offset 143.3 ms) occurred followed by iSP (onset 143.3 ms, offset 212.0 ms). The SP was interrupted by an increase in the EMG signal at about 166 ms. Following the iSP, there was a period of rebound EMG.

Figure 3
(A) iMEP threshold and cMEP thresholds for different current directions. Threshold values were measured at 50% maximal contraction for iMEP and 20% maximal contraction for cMEP. (B) iMEP and cMEP latencies for different current directions. iMEP were present in 8 out of 10 subjects but only 2 subjects had iMEPs in all four current directions. Error bars represent standard errors.

Figure 4
iSP threshold at different current directions. Thresholds were measured as ratios to the active contralateral FDI threshold (x AMT) and as absolute stimulation intensity (% of stimulator output). Error bars represent standard errors.
**Figure 5**

(A) Effects of stimulus intensities on iSP duration for different current directions. (B) Effects of stimulus intensities on iSP area for different current directions. Error bars represent standard errors.

**Figure 6**

Effects of current direction and interstimulus intervals (ISI) on interhemispheric inhibition. The conditioning stimuli applied to the right motor cortex at various ISI before the test stimulus to the left motor cortex. Test MEP amplitudes from the right FDI muscle were measured. Ratios less than one represent inhibition and ratios greater than one represent facilitation. Error bars represent standard errors. Inhibition was observed at ISIs of 8 to 50 ms and there was no significant effect of the current direction of conditioning stimuli.

**Figure 7**

(A) Effects of current direction and conditioning stimulus intensity on IHI at 8 ms ISI at rest. IHI increased with stimulus intensity up to 75% of stimulator output and there was no significant effect of current direction. (B) Effects of current direction and conditioning stimulus intensity on IHI at 40 ms ISI at rest. IHI increased with stimulus intensity up to 75% of stimulator output and there was no significant effect of current direction. (C) Effects of current direction and conditioning stimulus intensity on IHI at 8 ms ISI during target muscle activation. IHI was reduced compared to the rest condition shown in (A). (D) Effects of current direction and conditioning stimulus intensity on IHI at 40 ms ISI during target muscle activation. The extent of inhibition was similar to the rest condition (B). (E) Effects of current direction and conditioning stimulus intensity on cMEP amplitude. cMEP increased with stimulus intensity up to 75% of stimulator output and AM direction resulted in largest cMEPs. Error bars represent
standard errors.
References


Meyer BU, Roricht S, Schmierer K, Irlbacher K, Meierkord H, Niehaus L and Grosse P. First diagnostic applications of transcallosal inhibition in diseases affecting callosal neurones


Figure 1
Figure 3

A

Motor Threshold (% of Stimulator Output)

Direction of Induced Current

B

 MEP latency (ms)

Direction of Induced Current
Figure 4
Figure 5

A

ISP duration (ms)

Stimulus Intensity (% of Stimulator Output)

B

ISP area (mV.ms)

- O AM
- X PM
- ▲ PL
- △ AL

Figure 5
Figure 6