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The mechanisms of interhemispheric inhibition in the human motor cortex

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Transcranial magnetic stimulation can be used to non-invasively study inhibitory processes in the human motor cortex. Interhemispheric inhibition can be measured by applying a conditioning stimulus to the motor cortex resulting in inhibition of the contralateral motor cortex. Transcranial magnetic stimulation can also be used to demonstrate ipsilateral cortico-cortical inhibition in the motor cortex. At least two different ipsilateral cortico-cortical inhibitory processes have been identified: short interval intracortical inhibition and long interval intracortical inhibition. However, the relationship between interhemispheric inhibition and ipsilateral cortico-cortical inhibition remains unclear. This study examined the relationship between interhemispheric inhibition, short interval intracortical inhibition and long interval intracortical inhibition. First, the effect of test stimulus intensity on each inhibitory process was studied. Second, the effects of interhemispheric inhibition on short interval intracortical inhibition and long interval intracortical inhibition on interhemispheric inhibition were examined. Motor evoked potentials were recorded from the right first dorsal interosseous muscle in 11 right-handed healthy volunteers. For interhemispheric inhibition, conditioning stimuli were applied to the right motor cortex and test stimuli to the left motor cortex. For short interval intracortical inhibition and long interval intracortical inhibition, both conditioning stimuli and test stimuli were applied to the left motor cortex. With increasing test stimulus intensities, long interval intracortical inhibition and interhemispheric inhibition decreased, while short interval intracortical inhibition increased. Moreover, short interval intracortical inhibition was significantly reduced in the presence of interhemispheric inhibition. Interhemispheric inhibition was significantly reduced in the presence of long interval intracortical inhibition when matched for test motor evoked potential amplitude but the difference was not significant when matched for test pulse intensity. These findings suggest that both interhemispheric inhibition and long interval intracortical inhibition are predominately mediated by low threshold cortical neurons and may share common inhibitory mechanisms. In contrast, the mechanisms mediating short interval intracortical inhibition are probably different from those mediating long interval intracortical inhibition and interhemispheric inhibition although these systems appear to interact.

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Transcranial magnetic stimulation (TMS) has been used to demonstrate at least three different cortico-cortical inhibitory processes: interhemispheric inhibition (IHI), short interval intracortical inhibition (SICI) and long interval intracortical inhibition (LICI). IHI can be demonstrated by applying a conditioning stimulus (CS) to the motor cortex, which inhibits the size of the motor evoked potential (MEP) produced by the test stimulus (TS) of the opposite motor cortex (Ferbert *et al.* 1992; Hanajima *et al.* 2001). This result is consistent with animal studies that show stimulation of the motor cortex inhibits the contralateral motor cortex several milliseconds later (Chang, 1953; Asanuma & Okuda, 1962; Matsunami & Hamada, 1984). IHI can be observed at interstimulus intervals (ISIs) between 6 and 50 ms (Ferbert *et al.* 1992; Gerloff *et al.* 1998). Conversely, SICI and LICI are cortico-cortical inhibitory processes observed within the ipsilateral motor cortex. In the SICI paradigm, pairing a subthreshold CS with a suprathreshold TS at short ISIs (1–5 ms) inhibits the MEP produced by the TS (Kujirai *et al.* 1993). LICI results in attenuation of the MEP when a suprathreshold CS is paired with a suprathreshold TS at long ISIs (50–200 ms) (Valls-Sole *et al.* 1992; Wassermann *et al.* 1996).

Several lines of evidence suggest that these forms of cortico-cortical inhibition are mediated by cortical inhibitory neuronal mechanisms. For example, IHI is related to the

activity of inhibitory interneurons and largely mediated by transcallosal pathways. This contention is supported by several findings. First, test responses evoked by small anodal electrical shock are not significantly inhibited by contralateral magnetic conditioning stimuli (Ferbert *et al.* 1992; Hanajima *et al.* 2001). Low intensity electrical stimuli excite descending pyramidal axons within the white matter that are not sensitive to changes in cortical excitability (Rothwell, 1997). Second, H-reflexes in the relaxed forearm flexor muscles are unaffected by conditioning stimuli to the ipsilateral hemisphere, suggesting that ipsilateral motor cortex stimulation does not change spinal excitability (Ferbert *et al.* 1992; Gerloff *et al.* 1998). Finally, reduced excitability of the contralateral motor cortex has been demonstrated directly by recordings of descending corticospinal volleys (Di Lazzaro *et al.* 1999). Similarly, evidence that SICI and LICI are mediated by cortical inhibitory interneurons include: absence of any change in spinal excitability (Fuhr *et al.* 1991); failure to suppress the response to double transcranial electrical stimulation (TES; Ferbert *et al.* 1992; Inghilleri *et al.* 1993; Kujirai *et al.* 1993), and marked reduction in the corticospinal waves evoked by TMS (Valls-Sole *et al.* 1992; Nakamura *et al.* 1997; Chen *et al.* 1999).

Although these findings establish that IHI, SICI and LICI are all mediated by cortical inhibitory interneurons, other lines of evidence suggest that they are related to different subtypes of GABAergic receptors. For example, Sanger *et al.* (2001) found that SICI and LICI respond differentially to increasing TS intensities and that LICI inhibits SICI. Another important difference between SICI and LICI is that SICI is associated with a low intensity CS which produces shorter periods of cortical inhibition; whereas LICI is associated with a high intensity CS which produces longer periods of cortical inhibition. It is also known that $GABA_A$ receptor-mediated responses have lower activation thresholds and their inhibitory influence is brief (Davies *et al.* 1990; Sanger *et al.* 2001). Further, $GABA_B$ receptor-mediated responses have higher activation thresholds and their inhibitory influence is longer lasting (Deisz, 1999; Sanger *et al.* 2001). These findings have led researchers to suggest that SICI may be mediated by $GABA_A$ receptors while LICI may be mediated by $GABA_B$ receptors (Roick *et al.* 1993; Siebner *et al.* 1998; Werhahn *et al.* 1999).

Neurons mediating IHI must arise from contralateral sites and travel to the opposite hemisphere to exert their inhibitory effects. Since inhibitory GABAergic neurons mainly serve local circuits (Somogyi *et al.* 1998), IHI is probably mediated through excitatory axons that cross the corpus callosum to act on local inhibitory neurons in the contralateral motor cortex (Berlucci, 1990). However, it is currently unknown whether IHI is related to SICI and LICI and therefore mediated by similar or different GABAergic mechanisms.

One way to investigate whether experimental phenomena (i.e. SICI, LICI and IHI) share common mechanisms of action is to assess whether their profiles of response are similar or dissimilar under conditions of controlled perturbations. In these experiments, this is achieved in two ways; first, by a controlled manipulation of TS intensities on SICI, LICI and IHI and second by examining the impact of one inhibitory phenomenon on the other. This was accomplished by examining the interactions between SICI, LICI and IHI and intracortical facilitation (ICF) using a triple stimulation protocol. ICF was included because it may interact with these different inhibitory measures. Such methods have been used by Sanger *et al.* (2001) to examine the relationship between LICI and SICI. In the present experiments we use a similar approach to examine how IHI interacts with SICI and LICI. The findings will help us to understand how local inhibitory mechanisms are influenced by interhemispheric projections.

METHODS

Subjects

Three experiments were conducted. In Experiments 1 and 3 we studied 11 healthy, right-handed volunteers (mean age = 36.4 years, S.D. = 9.9 years, range = 26–57 years; 8 male and 3 female). In Expt 2 we studied 10 healthy, right-handed volunteers (mean age = 36.2 years, S.D. = 11.8 years, range = 23–57 years; 9 males and 1 female), eight of whom also participated in Experiments 1 and 3. Handedness was confirmed using the Oldfield Handedness Inventory (Oldfield, 1971). Subjects were recruited through advertisements in the community and postings within the hospital. All subjects gave their written informed consent and the protocol was approved by the University Health Network Research Ethics Board in accordance with the declaration of Helsinki on the use of human subjects in experiments. Exclusion criteria included a self-reported comorbid medical illness or a history of drug or alcohol abuse.

EMG recording

Surface EMG was recorded from the right and left first dorsal interosseous (FDI) muscles with disposable disc electrodes placed in a tendon-belly arrangement over the bulk of the FDI muscle and the first metacarpo-phalangeal joint. The subject maintained relaxation throughout the experiment and the EMG was monitored on a computer screen and via speakers at high gain. 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, UK) and stored in a laboratory computer for offline analysis.

TMS procedure

This study involved three experiments. The first experiment examined the effects of test MEP size on SICI, LICI, ICF and IHI. The second experiment examined the effects of IHI on SICI and ICF. The third experiment examined the effects of LICI on IHI.

TMS of the left motor cortex was performed with a 7 cm figure-of-eight coil and four Magstim 200 stimulators (The Magstim Company, Dyfed, UK) connected via three Bistim modules in a 'pyramid' setup. The output of each of the two pairs of Magstim 200 stimulators was connected to one Bistim module. The output

from the two Bistim modules was directed to a third Bistim module that was connected to the TMS coil. This setup allowed us to deliver up to four pulses of different stimulus intensities through the same coil at very short interstimulus intervals. The power attenuation of the pyramid system is about 15 %, similar to a single Bistim system (personal communication, Dr R. Jalinous, Magstim Company). The coil was placed at the optimal position for eliciting motor-evoked potentials (MEPs) from the right FDI muscle. The optimal position was marked on the scalp with a felt pen to ensure identical placement of the coil throughout the experiment. The handle of the coil pointed backwards and was perpendicular to the presumed direction of the central sulcus, about 45 deg to the midsagittal line. The direction of the induced current was from posterior to anterior and was optimal to activate the motor cortex transsynaptically (Werhahn *et al.* 1994; Kaneko *et al.* 1996).

TMS of the right motor cortex was performed with a 7 cm figure-of-eight coil and a Magstim Super Rapid stimulator. This stimulator produced bi-phasic current in the coil. The coil was placed at the optimal position for eliciting MEPs from the left FDI muscle. Stimulus intensity was set at 75 % of maximum stimulator output. The handle of the coil pointed forward and laterally about 45 deg to the midsagittal line. This orientation was chosen because in some subjects it was not possible to place both coils at the optimal positions with the handle pointed backwards and laterally due to the size of the coil. Previous studies in 11 normal subjects in our laboratory found no difference in the IHI between 75 % and 90 % of the stimulator output and between four coil orientations 90 deg apart (Yung & Chen, 2001).

This section explains the various parameters used in the experiments. The MT is expressed as a percentage of maximum stimulator output and was defined as the lowest intensity that produced MEPs of > 50 μV in at least five out of ten trials with the muscles relaxed. SICI and ICF were tested using paired-TMS with a subthreshold CS preceding a suprathreshold TS. CS2 denotes a conditioning stimulus that occurred 2 ms prior to a TS and CS10 denotes a conditioning stimulus that occurred 10 ms prior to a TS. CS2 was chosen because it consistently leads to SICI (Kujirai *et al.* 1993; Chen *et al.* 1998) and largely avoids the phenomenon of I-wave facilitation (Ziemann *et al.* 1998; Chen & Garg, 2000), which may obscure SICI (Awiszus *et al.* 1999). CS10 was chosen because it consistently gives rise to ICF (Kujirai *et al.* 1993; Ridding *et al.* 1995). LICI was tested with the suprathreshold CS and TS (Valls-Sole *et al.* 1992). The CS precedes the TS by 100 ms and is termed CS100. CS100 was used because at this interval direct recording of corticospinal waves demonstrated reduced cortical excitability (Nakamura *et al.* 1997; Chen *et al.* 1999) without any change in spinal excitability (Fuhr *et al.* 1991). IHI was tested with a suprathreshold CS delivered to the left motor cortex followed by a suprathreshold TS delivered to the right motor cortex 10 ms later. This CS will be referred to as CCS10 (contralateral conditioning stimulus). CCS10 was chosen because it consistently leads to IHI (Ferbert *et al.* 1992).

In all experiments the intensities of the TS were often adjusted to produce a target MEP size. An intensity of 'TS 1 mV' indicates a stimulator setting (determined to the nearest 1 % of the maximum stimulator output) that produces a peak-to-peak MEP amplitude of $\geqslant 1$ mV in at least 5 out of 10 trials. Similarly, 'TS 0.2 mV' and 'TS 4 mV' indicate settings that produce peak-to-peak MEP amplitudes of $\geqslant 0.2$ mV and $\geqslant 4$ mV in at least 5 out of 10 trials, respectively.

In Experiments 2 and 3, we compared the effects two inhibitory mechanisms together to that of one inhibitory mechanism alone. If we used the same test intensity throughout, the first inhibitory mechanism would decrease the test MEP amplitude upon which the second mechanism could operate. In order to match for test MEP amplitude, therefore, in some trials we increased the test stimulus intensity such that it would give a 1 mV test MEP in the presence of the first inhibitory mechanism. We then compared the effects of the second inhibitory mechanism on this 1 mV MEP to a 1 mV MEP that was elicited by a weaker single test pulse. Since both test MEP amplitude and test pulse intensity may be important in determining the degree of inhibition but it is not possible to match them at the same time, we designed our protocols to match for test MEP amplitude and test pulse intensity in different trials.

Experiment 1: effects of test stimulus intensity on SICI, ICF, LICI and IHI. In this experiment we examined the effects of different TS intensities on SICI, ICF, LICI and IHI. For SICI (CS2) and ICF (CS10), the intensity of the CS was set to 80 % of the MT (0.8 MT). For LICI the intensity of the suprathreshold CS100 was adjusted to produce a peak-to-peak MEP amplitude of about 1 mV and for IHI the CCS10 was set at 75 % of stimulator output. Each run consisted of 10 trials each of TS alone and four conditions with the conditioning stimulus preceding the test stimulus at different intervals (CS2, CS10, CS100, CCS10) delivered in random order. The time between trials was five seconds. Three TS intensities (TS 0.2 mV, TS 1 mV, and TS 4 mV) were studied in separate runs.

Experiment 2: effects of IHI on SICI and ICF. Here we investigated whether SICI and ICF are altered by IHI. Ten conditions were tested and are listed in Table 1 as 2*A*–2*J*. Each run consisted of 10 trials of each of the 10 conditions delivered in a random order (100 trials). Conditions 2*A*–2*D* were used to determine SICI, ICF and IHI for a 1 mV test MEP. Since IHI inhibits the test response, and SICI and ICF may be altered by an attenuated test MEP, for conditions 2*E*–2*J* the strength of the test stimulus was adjusted to produce 1 mV MEPs in the presence of an earlier CCS10 pulse. This test stimulus is referred to as 'TS 1 mV_{CCS10}'. This allowed us to match MEP amplitudes to produce a similar degree of corticospinal activation with and without preceding a CCS10. SICI and ICF in the presence of IHI were studied using three pulses in conditions 2*I* and 2*J*. We also measured SICI and ICF with the increased TS strength (TS 1 mV_{CCS10}) in conditions 2*F* and 2*G*. Therefore, we designed this experiment to compare SICI and ICF in the presence of IHI (2*I*/2*H* and 2*J*/2*H*) to SICI and ICF in the absence of IHI matched for test MEP amplitude (i.e. TS 1 mV; 2*B*/2*A* and 2*C*/2*A*) and TS intensity (i.e. TS 1 mV_{CCS10}; 2*F*/2*E* and 2*G*/2*E*).

Experiment 3: effects of LICI on IHI. In this experiment we investigated the effects of LICI on IHI. Seven conditions were tested and are listed in Table 1 as 3*A*–3*G*. Each run consisted of 10 trials of each of the 7 conditions delivered in a random order (70 trials). LICI and IHI for a 1 mV test MEP were determined from conditions 3*B* and 3*C*. Since IHI may be affected by test MEP amplitude and CS100 inhibits the test MEP, the strength of the test stimuli was adjusted to produce 1 mV MEPs in the presence of the CS100 pulse in conditions 3*D*–3*G*. This test pulse is referred to as 'TS 1 mV_{CS100}'. The interaction between IHI and LICI were studied using three pulses in condition 3*G*. Therefore, we designed this experiment to compare IHI in the presence of LICI (3*G*/3*F*) to IHI in the absence of LICI matched for test MEP amplitude (i.e. TS 1 mV) (3*B*/3*A*) and test stimulus intensity (i.e. TS 1 mV_{CS100}; 3*E*/3*D*).

Table 1. Stimulus conditions used in Expts 2 and 3

Condition	Stimulus intensity				
	CS100	CCS10	CS10	CS2	TS
2*A*	—	—	—	—	1 mV
2*B*	—	—	—	0.8MT	1 mV
2*C*	—	—	0.8MT	—	1 mV
2*D*	—	75 %	—	—	1 mV
2*E*	—	—	—	—	1 mV_{CCS10}
2*F*	—	—	—	0.8MT	1 mV_{CCS10}
2*G*	—	—	0.8MT	—	1 mV_{CCS10}
2*H*	—	75 %	—	—	1 mV_{CCS10}
2*I*	—	75 %	—	0.8MT	1 mV_{CCS10}
2*J*	—	75 %	0.8MT	—	1 mV_{CCS10}
3*A*	—	—	—	—	1 mV
3*B*	—	75 %	—	—	1 mV
3*C*	1 mV	—	—	—	1 mV
3*D*	—	—	—	—	1 mV_{CS100}
3*E*	—	75 %	—	—	1 mV_{CS100}
3*F*	1 mV	—	—	—	1 mV_{CS100}
3*G*	1 mV	75 %	—	—	1 mV_{CS100}

CS100, conditioning stimulus delivered 100 ms before TS (Expt 3); CCS10, contralateral conditioning stimulus delivered 10 ms before test stimulus; CS10, conditioning stimulus delivered 10 ms before TS (Expt 2); CS2, conditioning stimulus delivered 2 ms before TS (Expt 2); MT, resting motor threshold; TS, test stimulus. See Methods section for definition of TS intensity.

Data analysis

The peak-to-peak MEP amplitude for each trial was measured offline. Inhibition or facilitation was expressed as a ratio of the conditioned to mean unconditioned MEP amplitude for each subject. Ratios less than one indicate inhibition, and ratios greater than one indicate facilitation. Values are expressed as mean ± standard deviation (S.D.).

For Expt 1, the effects of TS intensity on SICI, LICI, ICF and IHI were evaluated by repeated-measures analysis of variance (ANOVA). If the effect of TS intensity was significant, Fisher's Protected Least Significant Difference (PLSD) *post hoc* test was used to detect differences among different TS intensities. Correlations between SICI and IHI were tested by Pearson product-moment correlation coefficients. In addition, it was found that the distribution for IHI values violated the assumptions of normality and homogeneity of variance and, therefore, was log transformed. For Expt 2, SICI and ICF alone at different test stimulus intensities (TS 1 mV and TS 1 mV_{CCS10}) and in the presence of IHI were compared using repeated-measures ANOVA. For Expt 3, IHI alone at different test stimulus intensities (TS 1 mV and TS 1 mV_{CS100}) and in the presence of LICI was compared using repeated-measures ANOVA. The threshold for significance was set at $P < 0.05$.

RESULTS

Experiment 1: effects of test stimulus intensity on SICI, ICF, LICI and IHI

The MEP amplitude for TS alone was 0.28 ± 0.13 mV for TS 0.2 mV, 0.87 ± 0.33 mV for TS 1 mV and 3.85 ± 2.18 mV for TS 4 mV. The results are shown in Fig. 1. Separate within-group repeated measures ANOVA demonstrated that increasing the TS intensity, from 0.2 mV to 4 mV, resulted in a significant decrease in IHI ($F = 18.91$, d.f. = 2, 20, $P < 0.001$) and LICI ($F = 8.90$, d.f. = 2, 20, $P = 0.002$) and a significant increase in SICI ($F = 4.65$, d.f. = 2, 20, $P = 0.02$). Increasing the TS intensity resulted in a small reduction in ICF, although this was not statistically significant. *Post hoc* testing showed that both IHI and LICI were significantly greater at 0.2 mV and 1 mV TS intensity than at 4 mV TS intensity. In contrast, SICI demonstrated little inhibition at 0.2 mV and but marked inhibition at 1 and 4 mV. There was no significant correlation between these measures of inhibition (i.e. SICI, LICI and IHI) at each TS intensity.

Experiment 2: effects of IHI on SICI and ICF

The MEP amplitude for TS 1 mV was 1.08 ± 0.49 mV (Table 1: condition 2*A*) and for TS 1 mV_{CCS10} was 2.42 ± 0.77 mV (2*E*). When a TS 1 mV_{CCS10} was preceded by CCS10 (2*H*), the test MEP amplitude was 1.05 ± 0.36 mV. Thus, the test MEP amplitudes for conditions 2*A* and 2*H* were matched. The IHI, SICI and ICF for a 1 mV test pulse was consistent with previous studies (Ferbert *et al.* 1992; Kujirai *et al.* 1993; Ziemann *et al.* 1996). Similar to the finding of Expt 1, IHI was higher with TS 1 mV (0.36 ± 0.05) compared to TS 1 mV_{CCS10} (0.49 ± 0.08, $P = 0.04$, paired *t* test). Figure 2 demonstrates the effects of combining CCS10 with CS2 in one representative subject and data for

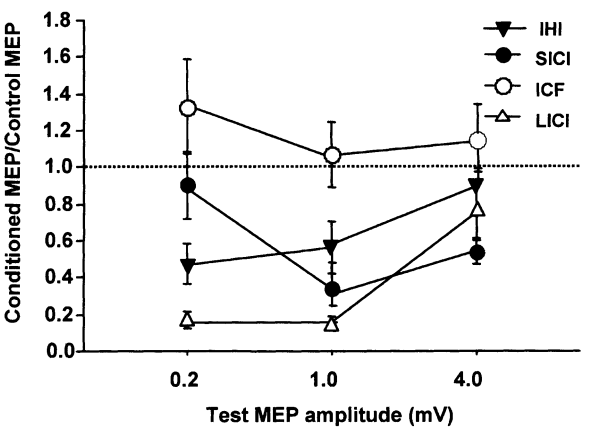

Figure 1. Effects of increasing TS intensity on cortical inhibition and facilitation

Data from 11 subjects. Each measure is expressed as a ratio (mean ± S.E.M.) of the conditioned MEP amplitude to the unconditioned MEP amplitude. Values below 1 indicate inhibition, greater than 1 indicate facilitation. With increasing TS intensity SICI increased whereas LICI and IHI decreased. ICF showed no significant change.

the entire sample are shown in Fig. 3. Compared to a TS alone (Fig. 2*A*), a preceding CS2 (Fig. 2*B*) or CCS10 (Fig. 2*C*) inhibited the test response. However, with CCS10 followed by CS2, there was little additional inhibition due to CS2 (Fig. 2*D*). The nature of the test MEP (TS 1 mV, TS 1 mV_{CCS10}, CCS10–TS 1 mV_{CCS10}; columns *A*, *B* and *C* in Fig. 3) had a significant effect on SICI ($F = 9.57$, d.f. = 2, 18, $P = 0.01$) (Fig. 3). *Post hoc* tests (PLSD) revealed a significant reduction in SICI in the presence of IHI compared to SICI alone when matched for either test MEP amplitude (i.e. TS 1 mV) (Fig. 3: *A vs. C*, $P = 0.004$) or test pulse intensity (i.e. TS 1 mV_{CCS10}; Fig. 3: *B vs. C*, $P = 0.0007$) whereas SICI for the two TS intensities were not significantly different (Fig. 3: *A vs. B*). Moreover, the change in SICI in the presence of IHI (calculated as a ratio of SICI in the presence of IHI to SICI alone) was greater in subjects with a stronger IHI and the correlation was significant ($r = 0.70$, d.f. = 2, 20, $P = 0.03$; Fig. 4*A*). In contrast, the change in SICI in the presence of IHI (calculated as a ratio of SICI in the presence of IHI to SICI alone) was not related to the strength of SICI ($r = 0.13$, d.f. = 2, 20, $P = 0.72$; Fig. 4*B*). ICF was not significantly affected by IHI (Fig. 3).

For each subject we also examined whether the inhibitory CS (CCS10 or CS2) became facilitatory in the presence of each other. In three subjects the CS2 pulse caused facilitation of the test MEP in the presence of the CCS10 pulse. That is, the MEPs of condition 2*J* (CCS10–CS2–TS 1 mV_{CCS10}) were larger than the MEPs of condition 2*H* (CCS10–TS 1 mV_{CCS10}). In one of these three subjects this facilitation was statistically significant ($t = 2.84$, d.f. = 9, $P = 0.02$). Similarly, in three subjects the CCS10 pulse caused facilitation of the test MEP in the presence of the CS2 pulse with the MEPs of condition 2*J* (CCS10–CS2–TS 1 mV_{CCS10}) larger than that of condition 2*F* (CS2–1 mV_{CCS10}). In two of the three subjects this facilitation was statistically significant ($t = 2.37$, d.f. = 9, $P = 0.04$ and $t = 2.26$, d.f. = 9, $P = 0.05$).

Experiment 3: effects of LICI on IHI

The IHI and LICI for TS 1 mV were consistent with previous studies (Ferbert *et al.* 1992; Chen *et al.* 1997). The mean MEP amplitude for TS 1 mV alone was 1.56 ± 0.55 mV (condition 3*A*) and for the TS 1 mV_{CS100} test pulse was 3.01 ± 1.38 mV. With a TS 1 mV_{CS100} preceded by CS100 (3*F*), the mean MEP amplitude was 1.44 ± 0.43 mV, similar to TS 1 mV alone (3*Aost-*). Consistent with the results of Expt 1, LICI was greater with TS 1 mV (0.18 ± 0.16) than with TS 1 mV_{CS100} (0.53 ± 0.22; $P = 0.002$, paired *t* test). IHI was also greater with TS 1 mV (0.44 ± 0.23) than with TS 1 mV_{CS100} (0.63 ± 0.20; $P = 0.014$, paired *t* test).

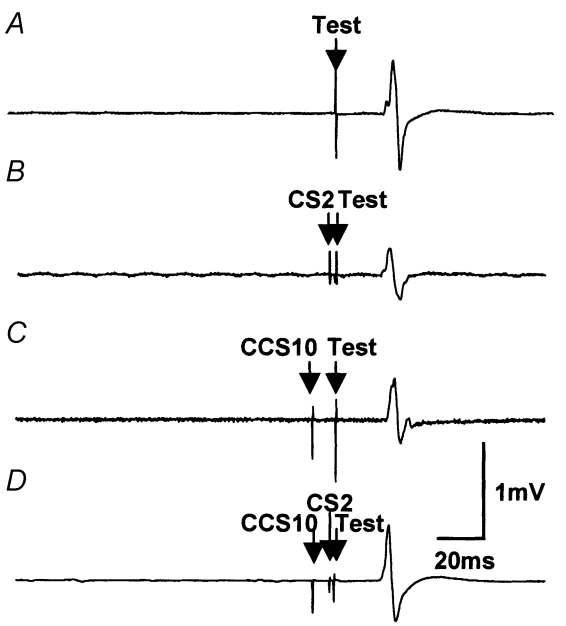

Figure 2. Effects of IHI on SICI in a single subject

These traces represent the averaged waveform form a single subject. In all traces the TS intensity was adjusted to produce 1 mV MEPs when preceded by a CCS10 (i.e. TS 1 mV_{CCS10}). *A*, response to TS 1 mV_{CCS10} alone (condition 2*E*). *B*, SICI alone: The conditioning stimulus (CS2) inhibited the test MEP (condition 2*F*) compared to *A*. *C*, IHI alone: The contralateral conditioning stimulus (CCS10) also inhibited the test response (condition 2*H*) compared to *A*. *D*, combined IHI and SICI: When the CCS10 preceded the CS2 (condition 2*I*), CS2 led to facilitation rather than inhibition of the test MEP compared to *C*.

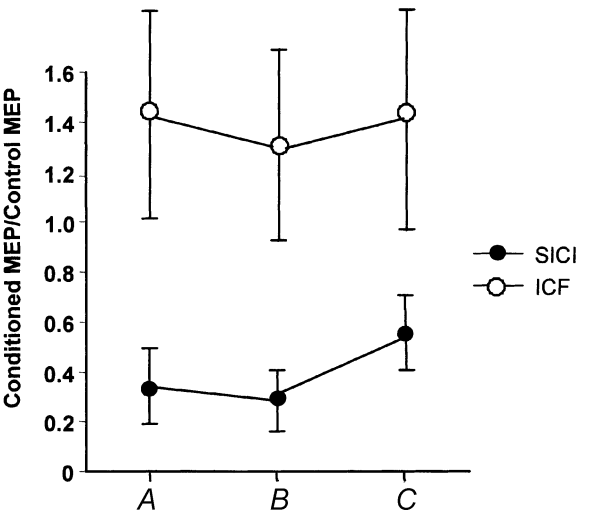

Figure 3. Effects of IHI on SICI and ICF

Data from 10 subjects. Both inhibition and facilitation are expressed as a ratio (mean ± S.E.M.) of the conditioned MEP amplitude to the unconditioned MEP amplitude. Values greater than one represent facilitation whereas values less than one represent inhibition. Points above *A* represent SICI and ICF using a TS that evokes a 1 mV MEP (i.e. TS 1 mV) (conditions 2*B*/2*A* and 2*C*/2*A*) and points above *B* represent SICI and ICF with a TS that evokes a 1 mV MEP if preceded by a CCS10 stimulus (i.e. TS 1 mV_{CCS10}) (condition 2*F*/2*E* and 2*G*/2*E*). Points above *C* demonstrate the triple stimulus approach in which a CS2 or CS10 are preceded by CCS10 (2*I*/2*H* and 2*J*/2*H*). Here the test stimulus was TS 1 mV_{CCS10} (condition 2*H*). There was significantly less SICI in the presence of IHI (*C*) compared to SICI in the absence of IHI (*A* and *B*). ICF was not significantly changed by IHI.

Figure 5 demonstrates the effects of combining a CS100 pulse with a CCS10 pulse in one representative subject. Compared to TS alone (Fig. 5*A*), a preceding CCS10 (Fig. 5*B*) inhibited the test response. In the presence of LICI, the CCS10 pulse no longer caused any inhibition (Fig. 5*D* compared to 5*C*). Data for the entire sample is shown in Fig. 6. The nature of the test MEP (TS 1 mV, TS 1 mV_{CS100}, CS100–TS 1 mV_{CS100}; columns *A*, *B* and *C* in Fig. 6) had a significant effect on IHI ($F = 27.98$, d.f. = 2, 18, $P = 0.0001$). Post-hoc tests (PLSD) revealed a significant reduction in IHI in the presence of LICI compared to IHI alone when matched for test MEP amplitude (i.e. TS 1 mV) (Fig. 6: *A vs. C*; $P = 0.002$) and a trend toward significance when matched for test pulse intensity (i.e. TS 1 mV_{CS100}) (Fig. 6: *B vs. C*; $P = 0.13$).

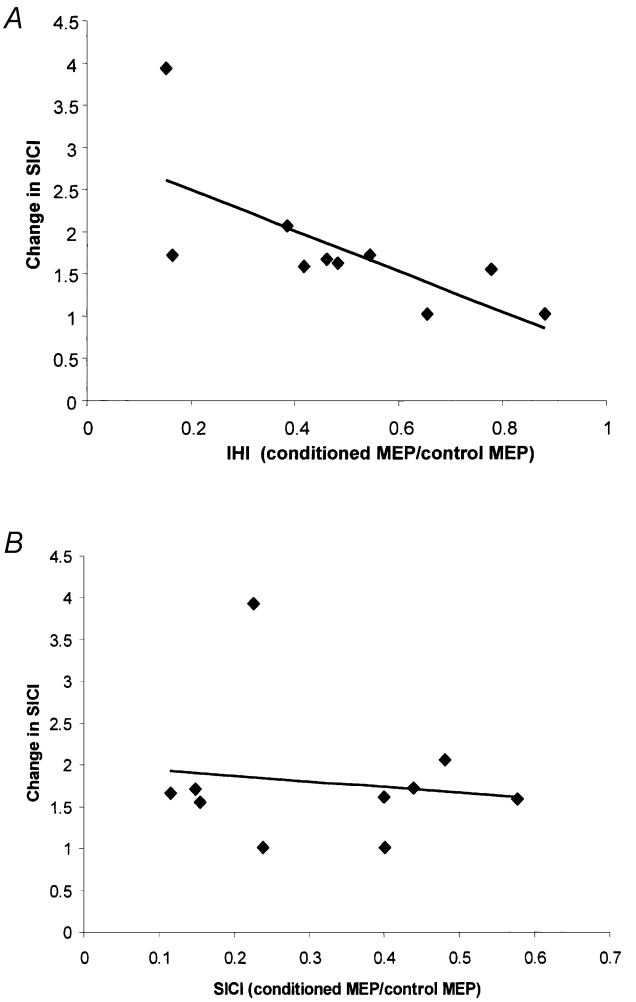

Figure 4. Effects of the strengths of IHI and SICI on IHI–SICI interaction

Data from 10 subjects and each point represents one subject. *A*, the relationship between IHI and the change in SICI in the presence of IHI. IHI is expressed as a ratio of the conditioned MEP amplitude to the unconditioned MEP amplitude (2*H*/2*E*). The *y*-axis represents a ratio of the SICI in the presence of IHI (2*I*/2*H*) to SICI alone (2*F*/2*E*). Change in SICI was significantly correlated with the strength of IHI. *B*, the relationship between SICI and the change in SICI in the presence of IHI. SICI is expressed as a ratio of the conditioned MEP amplitude to the unconditioned MEP amplitude (2*H*/2*E*). The *y*-axis represents a ratio of the SICI in the presence of IHI (2*I*/2*H*) to SICI alone (2*F*/2*E*). There was no correlation.

DISCUSSION

This study examined how IHI is related to SICI and LICI. In Expt 1, increasing TS intensities resulted in significantly less LICI and IHI but significantly greater SICI. Increasing TS intensity had no significant effect on ICF. In Expt 2 SICI was significantly reduced in the presence of IHI and this change in SICI was greater in subjects with stronger IHI. In Expt 3 IHI was significantly reduced in the presence of LICI when matched for test MEP amplitude but the difference was not significant when matched for test pulse intensity. A model that is consistent with our data is shown in Fig. 7.

Different neuronal populations mediate IHI and SICI

Changing the TS intensity had opposite effects on SICI and IHI. Similar to a previous study (Sanger *et al.* 2001), we found that SICI increases with higher test stimulus

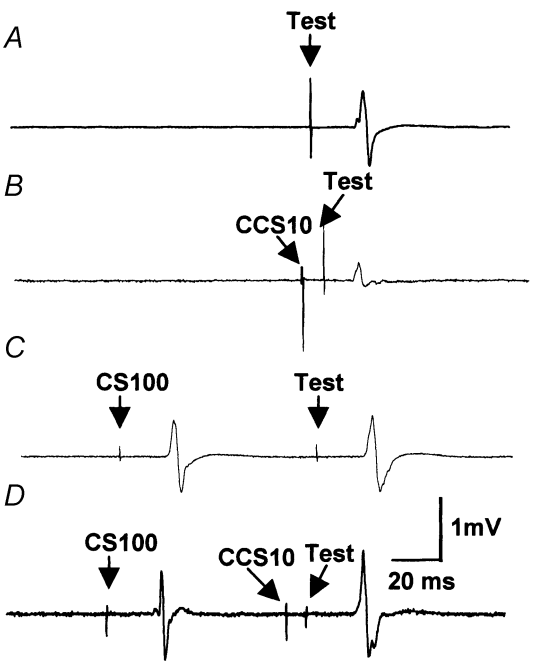

Figure 5. Effects of LICI on IHI in a single subject

Traces represent the averaged waveform for a single subject. *A*, response to TS 1 mV alone (condition 3*A*). *B*, IHI alone: A contralateral conditioning stimulus (CCS10) inhibited the test response (condition 3*B*) compared to *A*. The TS was the same as in *A*. C. LICI alone: A conditioning stimulus (CS100) using a TS that evokes a 1 mV MEP if preceded by a CS100 stimulus (i.e. TS 1 mV_{CS100}; condition 3*F*). The test MEP amplitude here is matched with that in *A*. *D*, combined LICI and IHI (condition 3*G*): In the presence of CS100, the CCS10 pulse caused no inhibition but a slight MEP facilitation compared to that shown in *C*.

intensity (i.e. 0.2 mV to 1 mV). In contrast, IHI decreases with increasing TS intensity, similar to the findings of Ferbert *et al.* (1992) in four subjects. These findings suggest that different neuronal circuits mediate IHI and SICI. The opposite effect of test MEP amplitude may be related to differences in activation thresholds or the location of cortical neurons mediating IHI and SICI. The cortical neurons mediating IHI may have lower activation thresholds than neurons mediating SICI. Alternatively, the neurons mediating IHI may be located at more superficial cortical layers than those mediating SICI. Another potential explanation to account for greater SICI with increasing TS intensities is related to the refractoriness of interneurons. The CS2 pulse may leave this inhibitory interneuron partially refractory to small test stimuli. Stronger test stimuli can overcome this refractoriness resulting in greater inhibition. Recent evidence suggests that there may be two phases of SICI with maximum inhibition at ISI of 1 ms and 2.5 ms (Fisher *et al.* 2002). Inhibition at ISI of 1 ms may be due to refractoriness whereas the inhibition as 2.5 ms is probably synaptic in origin. Since SICI at ISI of 2 ms is affected by GABAergic drugs (Ziemann *et al.* 1996) and voluntary activation (Ridding *et al.* 1995), it is more likely that this inhibition is predominately synaptic rather than due to neuronal refractoriness.

IHI inhibits SICI

In Expt 2 (Figs 2 and 3), we found that SICI was significantly reduced in the presence of IHI. Moreover, the extent of this reduction correlated with IHI but not SICI (Fig. 4*A* and *B*), suggesting that the effect is probably due to IHI inhibiting SICI rather than SICI inhibiting IHI. A potential confounding factor is that the various test conditions may preferentially activate different populations of cortical or spinal neurons with different susceptibility to IHI and SICI. Since IHI preferentially inhibits low threshold neurons (Fig. 1), the test MEP produced by the CCS10–TS 1 mV_{CCS10} combination (condition 2*H*) may largely be mediated by high threshold cortical neurons. However, this cannot explain our results because neurons activated at higher intensities are inhibited by SICI to a similar degree as those activated at lower intensities (Figs 1 and 3). The inhibition of SICI by IHI is similar to the inhibition of SICI by LICI demonstrated by Sanger *et al.* (2001). However, LICI almost completely abolished SICI (Sanger *et al.* 2001) while with IHI, SICI was reduced but still present (Fig. 3). The weaker inhibition of SICI by IHI compared to LICI may be related to the weaker MEP inhibition produced by IHI compared to LICI (Fig. 1).

Another potential explanation to account for decreased SICI in the presence of IHI is an occlusion or saturation effect. This may occur if the same or overlapping populations of inhibitory interneurons mediate SICI and IHI. In the presence of IHI, fewer inhibitory interneurons would be available to be activated by the SICI leading to reduced SICI. This cannot be completely excluded but several observations argue against this explanation. First, the difference in response of SICI and IHI to higher TS intensities (Fig. 1) argues against the suggestion that the same or overlapping neuronal population mediates SICI and IHI. Second, in one subject the CS2 pulse in the presence of IHI and in two subjects the CCS10 pulse in the presence of SICI caused significant MEP facilitation. The occlusion model cannot explain this facilitation. Third, the occlusion model predicts that subjects with greater IHI and SICI will have larger reduction of SICI in the presence of IHI. Although the change in SICI in the presence of IHI correlated with IHI (Fig. 4*A*), there was no correlation with SICI (Fig. 4*B*).

Similar neuronal populations may mediate IHI and LICI

LICI and IHI may share common mechanisms for several reasons. The first relates to the effects of different test stimulus intensities on LICI and IHI (Expt 1, Fig. 1). Both LICI and IHI decrease with increasing test stimulus intensity, suggesting that the neuronal pathways that mediate both inhibitory measures predominately act on motor cortical neurons activated at low intensities. Second, subthreshold conditioning stimuli are required to activate SICI inhibitory pathways, whereas suprathreshold conditioning stimuli

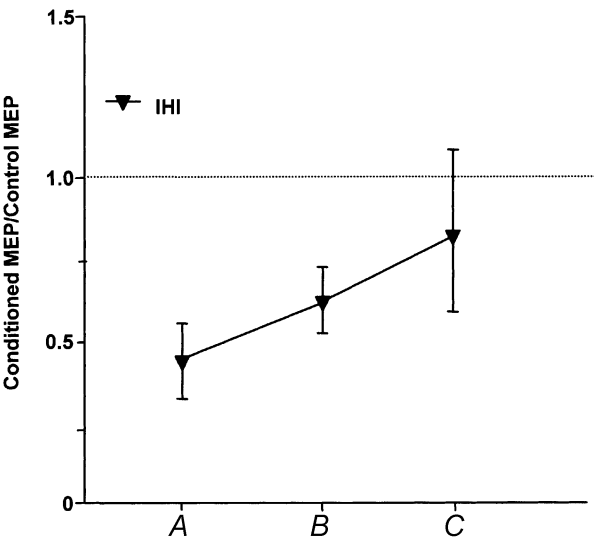

Figure 6. Effects of LICI on IHI

Data from 11 subjects. Inhibition is expressed as a ratio of the conditioned MEP amplitude to the unconditioned MEP amplitude (mean ± S.E.M.). Values less than one represent inhibition. *A*, IHI using a TS that evokes a 1 mV MEP (i.e. TS 1 mV) (condition 3*B*/3*A*). *B*, IHI using a TS that evokes a 1 mV MEP if preceded by a CS100 stimulus (i.e. TS 1 mV_{CS100}) (condition 3*E*/3*D*). *C*, the triple stimulus approach in which a CCS10 is preceded by a CS100 conditioning stimulus (3*G*/3*F*). Here the test stimulus was TS 1 mV_{CS100} (3*F*). IHI was less for the TS 1 mV_{CS100} (*B*) than the lower TS 1 mV (*A*). In the presence of LICI, IHI was significantly reduced when matched for TS 1 mV (*A vs. B*) but not when matched for TS 1 mV_{CS100} (*B vs. C*).

are required to activate LICI and IHI inhibitory pathways (Kujirai *et al.* 1993; Wassermann *et al.* 1996; Ziemann *et al.* 1996; Chen *et al.* 1998) suggesting that the activation thresholds for neurons mediating LICI and IHI are higher than the activation thresholds mediating SICI. A third line of evidence is the effect of voluntary contraction on these inhibitory paradigms. For both LICI (Valls-Sole *et al.* 1992; Wassermann *et al.* 1996) and IHI (Ferbert *et al.* 1992; Ridding *et al.* 2000) the extent of inhibition is similar at rest and during voluntary muscle contraction, whereas voluntary contraction markedly reduced SICI (Ridding *et al.* 1995). Fourth, both IHI and LICI inhibit SICI (Sanger *et al.* 2001). Differences also exist, however, between IHI and LICI. For example, the durations of IHI and LICI are different. LICI may last for 200 ms or longer depending on the conditioning stimulus intensity (Valls-Sole *et al.* 1992) while IHI last up to 50 ms (Gerloff *et al.* 1998; Yung & Chen, 2001). The longer duration of LICI compared to IHI may be related to stronger MEP inhibition elicited by LICI (Fig. 1). Nevertheless, these findings need to be confirmed in future studies.

Site of ipsilateral inhibition

Our results provide additional evidence that ipsilateral inhibition occurs at the cortical level (Ferbert *et al.* 1992; Di Lazzaro *et al.* 1999) in contrast to the findings of Gerloff *et al.* (1998) who suggested that ipsilateral inhibition may occur at subcortical sites and not necessarily though interhemispheric connections. Since both SICI (Kujirai *et al.* 1993; Nakamura *et al.* 1997; Di Lazzaro *et al.* 1998;) and LICI (Nakamura *et al.* 1997; Chen *et al.* 1999) are cortically mediated phenomena, our finding that contralateral motor cortex stimulation significantly influences SICI and LICI suggests that IHI occurs predominately at a cortical level.

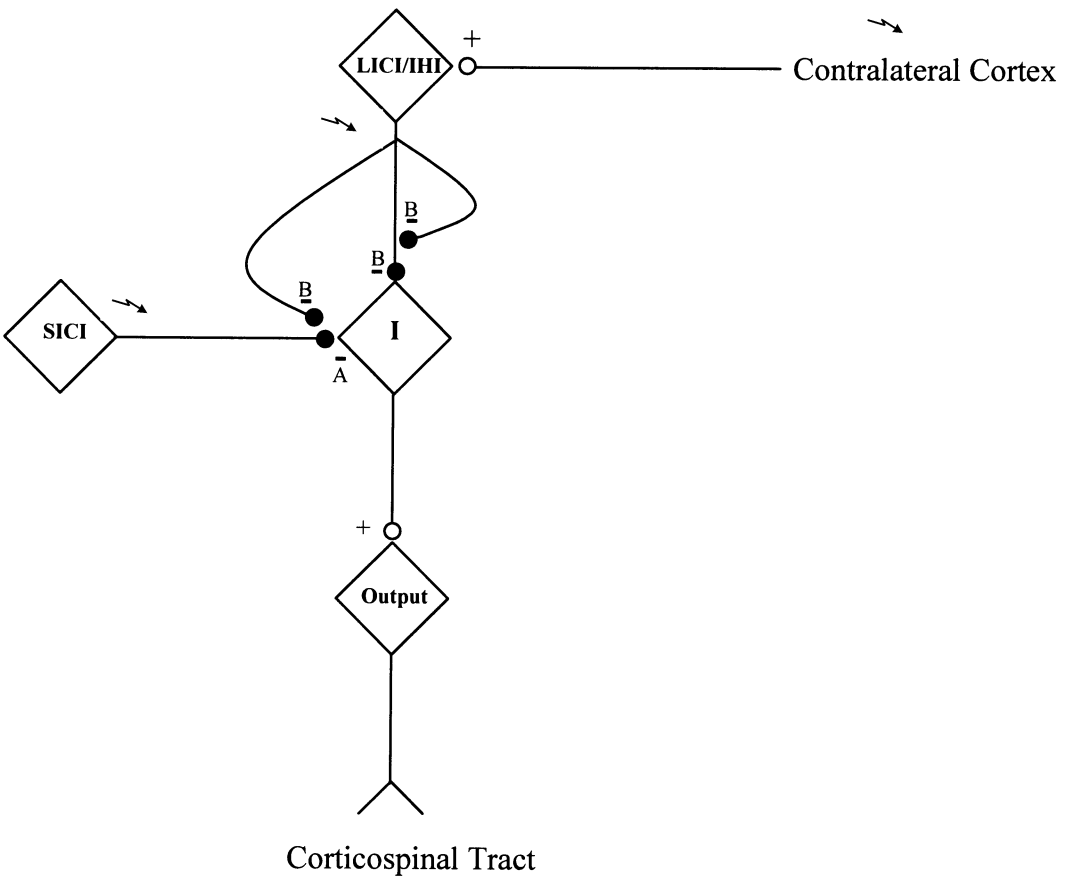

Figure 7. A hypothesis to explain our experimental findings

Each diamond schematically represents a population of neurons mediating the SICI, LICI, IHI or the motor response to test stimulus alone. The diamond labelled 'I' represents cells leading to descending I-waves and 'output' represents corticospinal output neurons. Filled circles represent inhibitory synapses and open circles represent excitatory synapses. 'Bolts' represent the presumed site of TMS stimulation. The letters 'A' and 'B' indicates the predominance of GABA receptor subtypes A or B. It is hypothesized that IHI is due to excitatory input from the contralateral motor cortex which activates inhibitory neurons that also mediate LICI (LICI/IHI). They cause MEP inhibition via postsynaptic $GABA_B$ receptors and cause auto-inhibition and inhibition of SICI via presynaptic $GABA_B$ receptors.

Possible role of GABAergic receptors

Both physiological and pharmacological studies have suggested that LICI may be mediated by $GABA_B$ receptors whereas SICI probably involve $GABA_A$ receptors (Roick *et al.* 1993; Siebner *et al.* 1998; Werhahn *et al.* 1999). Our results suggest that similar populations of inhibitory neurons may mediate LICI and IHI. Therefore, IHI may be related to $GABA_B$ activity. This is consistent with the finding that lorazepam increased SICI but did not change IHI, suggesting that IHI is not related to $GABA_A$ activity (Ziemann *et al.* 1996).

Interactions between LICI and IHI

In Expt 3 we found that IHI was reduced in the presence of LICI when matched for TS 1 mV, but this difference was not significant when matched for TS 1 mV_{CS100}. One explanation for this finding is that both IHI and LICI preferentially target low threshold cortical neurons. The MEPs produced by CS100–TS 1 mV_{CS100} (3*F*) pulse combination were probably mediated by higher threshold neurons than the MEPs produced by a test pulse alone (3*A*) even though they were matched for amplitude. Therefore, the reduced IHI in the presence of LICI (3*G*/3*F*) may be explained by these high threshold neurons being less sensitive to IHI. However, this is probably not the sole explanation. If LICI and IHI are mediated through an overlapping set of inhibitory neurons, the reduced inhibitory effects may be explained by a saturation effect. A third possibility is that the neurons mediating LICI and IHI inhibit one another. Single-cell recordings demonstrated a propensity for $GABA_B$ receptors to cause auto-inhibition (Rohrbacher *et al.* 1997). Therefore, cortical interneurons activated by LICI may cause auto-inhibition through $GABA_B$ presynaptic autoreceptors. Figure 7 depicts such as model. IHI may be mediated by contralateral excitatory inputs activating inhibitory interneurons that also mediate LICI. This hypothesis will have to be tested and refined in future studies.

In conclusion, IHI is associated with changes in the inhibitory circuits in the contralateral motor cortex. Both IHI and LICI are predominately mediated by low threshold cortical neurons and IHI may suppress the contralateral motor cortex through mechanisms similar to LICI. The mechanisms mediating SICI may be different from those mediating LICI and IHI although the finding that IHI inhibits SICI suggests that these mechanisms interact.

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