Handedness is mainly associated with an asymmetry of corticospinal excitability and not of transcallosal inhibition

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Abstract

Objective: The study aims to compare transcallosal inhibition (TI), as assessed by the paired-pulse transcranial magnetic stimulation (TMS) technique, in a sample of right-handed subjects (RH) and left-handed subjects (LH). Motor thresholds (MTs) and motor evoked potential (MEP) amplitudes were also measured in the two groups, as an index of corticospinal activity.

Methods: Thirty-two normal subjects (16 RH and 16 LH) were recorded with a paired-pulse TMS paradigm (intensity of both pulses = 120% of MT). The inter-stimulus intervals (ISIs) were 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 ms for both motor cortices, and MEP responses were recorded from the abductor digiti minimi muscles.

Results: Both groups showed a clear TI centred around the 12 ms ISI, but no difference was found as a function of handedness or of hemisphere. On the other hand, the two groups differed in terms of corticospinal activity, since the hand motor dominant hemisphere had lower MTs than the non-dominant one in LH, and larger MEP amplitudes for the right hand were found in RH.

Conclusions: Results point to a functional asymmetry of the motor cortex on the hand-dominant versus the non-dominant hemisphere, while handedness does not seem associated with functional differences in callosal inhibition, as measured by the inter-hemispheric paired-pulse TMS technique.

Keywords: Transcranial magnetic stimulation; Handedness; Paired-pulse technique; Transcallosal inhibition; Corpus callosum; Corticospinal system; Gender

1. Introduction

Several studies in recent years have assessed the association between handedness and inter-hemispheric connectivity. Volumetric measurements found a larger corpus callosum (CC) in left-handers compared to right-handers (Clarke and Zaidel, 1994; Cowell et al., 1993; Denenberg et al., 1991; Habib et al., 1991; Luders et al., 2003; Moffat et al., 1998; Preuss et al., 2002). The size of the CC has been related to the number of myelinated and non-myelinated fibres involved in the transmission of neuronal activity (Aboitiz et al., 1992), to functional cortical activation assessed by fMRI during uni- and bi-manual movements (Stancak et al., 2003), and to hemispheric speech representation (Moffat et al., 1998).

Considering the uncertainty of dividing the CC into subregions (e.g. Peters et al., 2002), the existence of CC connections between the homotopic and heterotopic cortical regions (e.g. Seltzer and Pandya, 1983), the sparse callosal fibres connecting M1 areas (Rouiller et al., 1994), and the lack of a direct physiological measure of CC activity in humans, it is not surprising that the relationship between CC morphology and motor activity in handedness groups has not been established once and for all.

A measure of CC activity in humans is now provided by a recent transcranial magnetic stimulation (TMS) paradigm using inter-hemispheric paired pulses. TMS is now an...
established technique in cognitive neurosciences (Walsh and Cowey, 2000) allowing, besides many other applications, the non-invasive assessment of motor cortical excitability (Rossini et al., 1999). The inter-hemispheric paired pulse paradigm uses two magnetic stimulators investigating the effect of a conditioning magnetic stimulus over the motor cortex of one hemisphere on the size of EMG responses evoked in the muscle by a magnetic test stimulus given over the opposite hemisphere (Ferbert et al., 1992). Early experiments showed how the magnetic stimulation of one hemisphere’s motor cortex leads to the inhibition of the EMG response after a second stimulation in the contralateral motor cortex delivered a few milliseconds later (Hanajima et al., 2001; Ferbert et al., 1992). Although inhibitory interactions may be mediated via transcallosal connections, it is very likely that also subcortical mechanisms are involved (Gerloff et al., 1998); in fact, cortico-reticulo-spinal and propriospinal pathways could also contribute to ‘inter-hemispheric’ interaction. Meanwhile, along the vein focusing on the CC, Ferbert et al. (1992) firstly hypothesized that the inhibition is produced at a cortical level via a transcallosal route, mainly on the basis of the finding that the inter-stimulus interval (ISI) of this inhibition phenomenon is coherent with the estimates of the callosal conduction time found with other methods (Cracco et al., 1989; Saron and Davidson, 1989). The hypothesis of a transcallosal origin of the observed inhibition (TI) is supported by its impairment in patients with agenesis of the CC, and with total or partial callosotomy (Meyer et al., 1995, 1998; Moffat et al., 1998; Netz et al., 1995). A direct demonstration was also provided in patients with epidural spinal cord electrodes for relief of intractable pain (Di Lazzaro et al., 1999). Recordings of the descending spinal volleys directly from the epidural space of the cervical cord showed a reduction in the size of the I-waves by a prior conditioning stimulus to the opposite hemisphere (Di Lazzaro et al., 1999).

The role of transcallosal fibres in the control of unilateral movements in subjects with different hand preference has indeed been assessed by Netz et al. (1995), who measured TI at a the 10 ms ISI in a sample of normal right-handers (RH) and left-handers (LH). A transcallosal inhibition was found only in RH, while LH showed an inhomogeneous pattern of results that the authors put down to language hemispheric dominance instead of motor dominance. In fact, their use of a single interval between conditioning and test stimuli precluded any detection of the existence of TI in the full range of ISIs usually considered in these experiments. In other words, transcallosal connectivity could not have been assessed appropriately in those subjects showing motor inhibition at ISIs different from 10 ms.

Within this theoretical framework the aim of the present study is thus to assess the full range of ISIs characterising the amount and direction of TI with the paired-pulse, inter-hemispheric TMS technique in an adequate sample of right- and left-handed subjects. Furthermore, since in a recent study females showed a higher transcallosal inhibition at the 12 ms ISI (De Gennaro et al., 2004), gender differences were also evaluated along with the interactions between gender and handedness. Finally, parameters reflecting corticospinal excitability were measured as a function of handedness.

2. Methods and materials

2.1. Subjects

Sixteen males and sixteen females [mean age = 25.94 years (SE = 0.79)] were selected from a university student population. Hand preference was measured by a standard handedness questionnaire (Salmaso and Longoni, 1985). Handedness Scores (HS) ranged from 1 to −1, where 1 defined the maximum of dexterity. The criteria employed to select right-handers (RH) and left-handers (LH) were, respectively, HS ≥ 0.70 and HS ≤ −0.70. Sixteen subjects (8 M and 8 F) were selected as RH [HS = 0.86 (SE = 0.04)] and another sixteen (8 M and 8 F) as LH [HS = −0.83 (SE = 0.04)]. In a clinical interview, the subjects reported the absence of epilepsy or of any other neurological and psychiatric condition in themselves and in their known family history. The protocol of the study was approved by the appropriate Institutional Ethics Committee and the subjects gave their written informed consent, according to the Declaration of Helsinki.

2.2. Materials

The stimulators used were two Magstim 200 Mono Pulses connected to a Bistim module and to two figure-of-eight coils with an external wing diameter of 9 cm (Magstim Company Limited, UK). The Bistim module was used to regulate the sequence of conditioning and test pulses at the different ISIs. The peak magnetic field produced by these coils was 2.0 T.

The motor evoked potentials (MEPs) of the hand muscles were recorded from the abductor digiti minimi (ADM) muscles, in both hands. Two Ag–AgCl surface cup electrodes of 9 mm diameter were used: the active electrode was placed over the muscle belly, while the reference electrode over the metacarpophalangeal joint of the little finger. The MEP were recorded according to standard procedures (Rossini et al., 1994, 1999), and were stored and analysed with an EMG-dedicated software (Myto, EBNeuro, Italy).

2.3. Procedure

During the experiment, the subjects sat fully relaxed on a comfortable chair, with eyes open and watching a point on
the wall. The paired-pulse paradigm allowed us to assess the effect of a conditioning stimulus delivered on the motor cortex of one hemisphere, and on the MEP amplitude evoked in the ADM muscles by a magnetic test stimulus applied to the opposite homologous cortex. Several inter-stimulus intervals (ISIs) were used between the conditioning and test magnetic pulses. Twelve responses per condition, both test and conditioning pulses, were collected and their peak-to-peak amplitude was measured off-line and subsequently averaged. The positions of the two coils were kept constant throughout each block of stimulations; no difficulties were encountered in allocating both coils on the head and maintaining them in a stable position throughout the experimental session.

The most effective point on the subject’s scalp for eliciting a target muscle stimulation was localized by positioning the coil such that the junction region of the figure-of-eight coil was approximately over the central sulcus and by moving the coil in 1 cm steps. The coils were positioned tangentially to the scalp oriented in a postero-anterior direction, 45° from the midsagittal axis of the subject’s head. In this way, the induced current in the brain flows in the anterior–posterior direction. This orientation was chosen because stronger effects of inter-hemispheric inhibition were found when the conditioning stimulus induced posteriorly directed currents (Hanajima et al., 2001) and because this direction is the most effective for eliciting transcallosal inhibition (Meyer et al., 1995).

A test session was then carried out in order to establish the resting motor threshold (MT) of excitability for each subject. The MT was established via a standardized method (Rossini et al., 1994, 1999), as the lowest intensity level of stimulation able to produce at least 3 MEPs with 100 μV of amplitude (peak-to-peak) in 6 consecutive stimulations. These procedures were repeated for both hemispheres.

The stimulation intensity was set at 120% of the individual MT for both CS and TS, because early experiments showed that a suprathreshold CS intensity is more effective in reducing the MEP elicited by the TS. In fact, a subthreshold CS at 80% of the individual MT does not affect responses to TS delivered to each cerebral hemisphere (De Gennaro et al., 2004).

The effect of the conditioning pulse in reducing MEP amplitude was assessed in the following ISIs: 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 ms. The baseline level of MEP responses (unconditioned responses) was measured with an independent series of test stimuli, administered alone at 120% of the individual MT. Also for unconditioned responses, to avoid uncontrolled intervening factors, both coils were positioned on the subject’s scalp with the same orientation as the conditioned responses. Each recording session included 22 blocks [(10 conditioned blocks with different ISIs + 1 baseline) × two hemispheres]. These blocks were partially balanced between subjects. Since a complete counterbalancing of the sequences would have required a larger number of subjects in order to control for any possible sequence effect and for any modification of the excitability thresholds across the testing period, we considered 8 sequences in which 5 blocks of adjacent ISIs and the baseline for each hemisphere were administered in the beginning or the middle or the end of the session. Half the subjects started with the right hemisphere and the other half with left one. Identical sequences were used in the 4 Gender × Handedness groups.

2.4. Data analysis

The resting MTs were analysed by means of a mixed design analysis of variance (ANOVA), Gender × Handedness × Hemisphere, in order to compare the inter-hemispheric differences between the subgroups (RH, LH, females and males).

Amplitudes were measured both as baseline to the first reproducible peak and as peak-to-peak between the two major ones. The mean MEP amplitude of unconditioned responses (test alone) was submitted to the same ANOVA design (Gender × Handedness × Hemisphere). Interactions between MTs, baseline MEP amplitudes and the handedness score were assessed by Pearson’s correlations.

Changes in MEP amplitude as a consequence of conditioning pulse administration were expressed in terms of the ratio between test responses (preceded by conditioning pulses) divided by unconditioned responses (in which only the test pulse was administered). To assess the influence of gender and handedness on TI at different ISIs, changes in MEP amplitude were considered as a dependent variable in a repeated measure ANOVA Gender × Handedness × ISI × Hemisphere. To correct for these multiple comparisons, the Bonferroni correction was applied. Taking into account the mean correlation between the variables considered in these comparisons, the alpha level was adjusted to ≤0.01.
3. Results

3.1. Corticospinal excitability

The mean MT at rest was 37.46% (SE = 1.16) of the maximum output of the magnetic stimulator for the right and 39.46% (SE = 1.47) for the left hemisphere.

The ANOVA on mean MTs yielded no significant effect but a significant Handedness × Hemisphere interaction ($F_{1,28} = 5.61, P = 0.02$), mainly explained by lower MTs for the right hemisphere than for the left one in left-handers ($P < 0.05$ at the planned comparison). On the other hand, right-handers had lower MTs in the left hemisphere compared to the right one, although this difference was not significant (Fig. 1).

The same analysis on the MEP amplitude of unconditioned responses showed an effect for Handedness ($F_{1,28} = 16.82, P = 0.0003$) with higher MEP amplitudes in right-handers [1481.03 V (SE = 26.83)] than in left-handers [857.94 V (SE = 91.67)], and again a significant Handedness × Hemisphere interaction ($F_{1,28} = 4.03, P = 0.05$). The differences between the means of this interaction are consistent with those of MTs (Fig. 2), although in this case the significant difference concerned right-handers ($P < 0.05$ at the planned comparison).

Handedness scores were not significantly correlated to both MTs and baseline MEP amplitudes, either for right- or left-handers. Only the MTs of right and left motor cortices showed a significant correlation in both groups (Table 1).

3.2. Transcallosal inhibition

The ANOVA on MEP ratios showed only a significant effect for the ISI factor ($F_{9,252} = 8.04, P < 0.00000001$). No other main effect or interactions were significant. More specifically, the Gender × ISI interaction was not significant ($F_{9,252} = 0.89, P = 0.54$), and the Handedness × ISI interaction also pointed to the lack of any difference of the values of transcallosal inhibition at each ISI between the two groups ($F_{9,252} = 0.43, P = 0.92$). Both handedness groups showed a similar transcallosal inhibition that was actually present. This inhibition is clearly discernible in Fig. 3, which also reports the significance of MEP changes against the null hypothesis of test responses = unconditioned responses: right-handers showed significant MEP reductions in the 6–16 ms ISI range and left-handers in the 8–16 ms range ($P < 0.01$), while no MEP change was significant in both handedness groups for the ratio between MEP responses to conditioning pulses and unconditioned responses.

Similarly, changes in MEP amplitude did not discriminate between the right and left motor cortex of the two handedness groups, with no ‘Handedness × ISI × Hemisphere’ interaction ($F_{9,252} = 0.79, P = 0.62$). Left-handers showed the same pattern of MEP amplitude reductions with a maximum at the 12 ms ISI for both motor cortices; similar proportions of MEP amplitude reduction were also found in right-handers, with the largest inhibition at the 10 ms ISI, when the conditioning pulse was delivered to the left hemisphere, and at 12–14 ms, when it was delivered to the right hemisphere.

To further assess the differences between the two handedness groups, the data were plotted in terms of
the dominant hand motor areas for both groups, and the two groups were compared for each ISI by two-tailed Student’s t tests for independent samples. No comparison between right- and left-handers was significant at any ISI when conditioning pulses were delivered to the dominant or to the non-dominant motor cortex (Fig. 4).

A greater asymmetry in right – as compared to left-handers, as previously reported using only a 10 ms ISI (Netz et al., 1995), was not found – even when taking individual differences into consideration (Fig. 5). The degree of inter-hemispheric inhibition at 10 ms also did not correlate with handedness score in right- (r = 0.02, P = 0.93) and left-handers (r = 0.31, P = 0.25).

Regardless of handedness and hemisphere, gender influence on transcallosal inhibition at the specific 12 ms ISI was also analysed, since a larger TI had been found in females at this ISI (De Gennaro et al., 2004). As shown in Fig. 6, the significant difference confirms the higher MEP change in females (66.00%, SE = 2.70) with respect to males (77.64%, SE = 4.59; t30 = 2.19, P = 0.04) at this ISI.

Table 1
Inter-correlations among handedness scores, motor thresholds (MTs) and baseline motor evoked potentials (MEP) amplitudes

<table>
<thead>
<tr>
<th></th>
<th>MT (right motor cortex)</th>
<th>MT (left motor cortex)</th>
<th>Baseline MEP (right motor cortex)</th>
<th>Baseline MEP (left motor cortex)</th>
</tr>
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<tbody>
<tr>
<td><strong>Right-handers</strong></td>
<td></td>
<td></td>
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<tr>
<td>Handedness score</td>
<td>0.46 (P = 0.07)</td>
<td>-0.21 (P = 0.43)</td>
<td>-0.27 (P = 0.32)</td>
<td>0.17 (P = 0.53)</td>
</tr>
<tr>
<td>MT (right motor cortex)</td>
<td>0.74 (P = 0.001)</td>
<td>-0.18 (P = 0.51)</td>
<td>-0.38 (P = 0.15)</td>
<td></td>
</tr>
<tr>
<td>MT (left motor cortex)</td>
<td></td>
<td>-0.28 (P = 0.30)</td>
<td>-0.37 (P = 0.16)</td>
<td></td>
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<tr>
<td>Baseline MEP (right motor cortex)</td>
<td></td>
<td></td>
<td>0.43 (P = 0.09)</td>
<td></td>
</tr>
<tr>
<td><strong>Left-handers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Handedness score</td>
<td>-0.11 (P = 0.68)</td>
<td>0.15 (P = 0.57)</td>
<td>0.17 (P = 0.53)</td>
<td>-0.34 (P = 0.20)</td>
</tr>
<tr>
<td>MT (right motor cortex)</td>
<td>0.80 (P = 0.0002)</td>
<td>-0.07 (P = 0.80)</td>
<td>-0.08 (P = 0.78)</td>
<td></td>
</tr>
<tr>
<td>MT (left motor cortex)</td>
<td></td>
<td>0.02 (P = 0.95)</td>
<td>-0.05 (P = 0.86)</td>
<td></td>
</tr>
<tr>
<td>Baseline MEP (right motor cortex)</td>
<td></td>
<td></td>
<td>0.31 (P = 0.24)</td>
<td></td>
</tr>
</tbody>
</table>

Coefficients significant at P ≤ 0.001 level are italicised.

Fig. 3. Mean changes in MEP amplitude (and standard errors) of conditioning and test responses at different inter-stimulus intervals (ISI) for both right- and left-handers. The asterisks point to the significance of MEP reduction, assessed by means of a one-sample Student’s t test against the null hypothesis of a population mean ratio equal to 1 (conditioned responses = unconditioned responses). The shaded area highlights the ISI range in which transcallosal inhibition was found. **P ≤ 0.01; ***P ≤ 0.001.
4. Discussion

Suprathreshold magnetic conditioning stimuli on one hemisphere reduced the MEP amplitude of the ADM contralateral to the other hemisphere, which was stimulated at specific time intervals (test stimulus). The range of these intervals is consistent with the range reported by studies using the inter-hemispheric paired pulse paradigm (Ferbert et al., 1992; Ridding et al., 2000; Sakai et al., 1998; Ziemann et al., 1996), and

Fig. 4. Mean changes in MEP amplitude (and standard errors) of test responses at different inter-stimulus intervals (ISI) for both right- and left-handers, as a function of the dominant hand motor areas.

Fig. 5. Individual differences in handedness groups in hemispheric asymmetry in transcallosal inhibition at the 10 ms ISI. The asymmetry index was computed according to the following formula: \((\text{left} - \text{right})/(\text{left} + \text{right})\). Right and left indicate the side of the conditioned motor cortex.

Fig. 6. Mean changes in MEP amplitude (and standard errors) of test responses at different inter-stimulus intervals (ISI) in male and female subjects. The shaded area points to a greater MEP reduction in females than in males at the 12 ms ISI, as assessed by the Student’s \(t\) test.
estimates of callosal conduction time (8.8–12.2 ms) obtained with focal electrical or magnetic stimulation of the frontal cortex and recording the onset of responses over the contralateral cortex (Aboitiz et al., 1992; Cracco et al., 1989; Rossini et al., 1985). However, subjects with different hand preference do not show a different transcallosal inhibition, since a 33.75% average MEP reduction was found in right-handers at the significant 6–16 ms ISI range, and a 32.00% reduction in left-handers at the 8–16 ms ISI range. This is in disagreement with the previous study by Netz et al. (1995), who found differences in TI comparing RH and LH; a probable explanation for this is that the examination was carried out during tonic contractions and that only one ISI at 10 ms was tested.

The callosal fibres connecting the right and left motor (M1) and supplementary (SMA) motor cortices are involved in the control of bimanual coordination (Gerloff and Andres, 2002; Stancak et al., 2003). According to the current results, this control does not seem to be affected by hand preference. In fact, most morphological studies showing a larger size of the CC in left-handers mainly point to differences in the callosal isthmus (Clarke and Zaidel, 1994; Cowell et al., 1993; Habib et al., 1991; Moffat et al., 1998; Witelson, 1989), while callosal subregions containing fibres connecting motor cortices do not show significant differences related to motor dominance (Moffat et al., 1998; Peters et al., 2002).

Regardless of handedness, the gender difference observed at the 12 ms ISI confirms the higher transcallosal inhibition in females with respect to males at this ISI (De Gennaro et al., 2004).

While hand preference does not affect TI, right- and left-handers indeed differ with respect to MTs and MEP amplitude of unconditioned pulses. Results show that the hand motor dominant hemisphere has lower MTs than the non-dominant one, mainly in left-handers. This finding is in line with most studies comparing MTs in different handedness groups (Cantello et al., 1992; Macdonell et al., 1991; Netz et al., 1995; Triggs et al., 1994, 1997), although other studies with smaller samples failed to show any significant difference (Cicinelli et al., 2000; Civardi et al., 2000). Similarly, our differences in MEP amplitudes in response to unconditioned pulses go in the same direction: right-handers showed significantly greater MEPs for the right hand while left-handers showed a tendency in the opposite direction, confirming previous observations (Netz et al., 1995).

It should, however, be recalled that changes in MTs and in MEP amplitude reflect the excitability of the corticospinal system, and the exact locus of these changes in excitability along the corticospinal pathway might be determined by different methods (evaluation of spinal and subcortical excitability, e.g. H-reflex, F-wave, transcranial electric stimulation, electric brain-stem stimulation) which, however, were unsuitable for the present, already long-lasting, experimental session. In fact, the full collaboration of cooperative subjects—mainly in maintaining the upper limb and hand muscles on both sides completely relaxed—was pivotal for the success of the protocol. Adding more experiments would have made these objectives more difficult to achieve.

On the other hand, motor mapping TMS studies and cortico-cortical paired pulse TMS studies provide a further and more direct support to the hypothesis of a greater excitability in the motor cortex contralateral to the dominant hand. As a matter of fact, a larger cortical representation of the right abductor pollicis brevis (APB) was found in right-handers, and of the left APB in left-handers (Cantello et al., 1992; Krings et al., 1997; Triggs et al., 1999). Moreover, the former showed more cortico-cortical facilitation and less cortico-cortical inhibition in the left than in the right motor cortex (Civardi et al., 2000).

Therefore, measurements of MTs and of MEP amplitudes, TMS mapping of motor cortical representations and the cortico-cortical paired-pulse TMS paradigm converge in pointing to a functional asymmetry of the motor cortex on the hand-dominant versus the non-dominant hemisphere. This association between a motor system asymmetry and hand preference is also confirmed by animal studies. Distal forelimb movement cortical representations derived from intracortical microstimulation in adult squirrel monkeys are greater in number and larger in total area in the dominant hemisphere, that is the hemisphere opposite the preferred hand (Nudo et al., 1992).

In conclusion, our study shows an association between a corticospinal asymmetry and hand preference in which there is, at least, a contribution of the cortical component of the motor system. On the other hand, in the present experimental conditions, handedness does not seem to be associated with functional differences in callosal activity as measured by the inter-hemispheric paired-pulse TMS technique. However, we are aware that with this study, functional differences, as measured by the inter-hemispheric paired-pulse TMS technique, are only partially assessed and further aspects of inter-hemispheric connections should be addressed (Chen et al., 2003; Hanajima et al., 2001; Netz et al., 1995).

References


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