Changes in effective connectivity of the primary motor cortex in stroke patients after rehabilitative therapy

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Received 11 November 2005; revised 16 February 2006; accepted 23 April 2006
Available online 7 July 2006

Abstract

We used a perturb-and-measure approach, by combining transcranial magnetic stimulation (TMS) and positron emission tomography (PET), to examine changes in the primary motor area (M1) and its effective connectivity in stroke patients with chronic motor deficits (>1-year post-stroke) who underwent 3 weeks of constraint-induced movement therapy. During the 3-week period, 7 patients spent 4 h per day performing shaping exercises with the affected arm under our supervision for 14 days and wore a mitt on the unaffected arm at home in situations where safety was not compromised. Anatomical magnetic resonance imaging confirmed that all patients had lesions that encompassed the white matter; no patient had damage in the hand representation of M1. Improvements on various motor tests were observed immediately after therapy and 1 month afterwards. During the TMS/PET sessions, we applied trains of subthreshold 10-Hz repetitive TMS over the hand representation of the ipsilesional and contralesional M1s and varied the number of TMS trains delivered during each scan. The results demonstrate changes in the local response of TMS in the ipsilesional and contralesional M1, changes in the strength of interhemispheric connectivity between M1s, and changes in the effective connectivity of the ipsilesional and contralesional M1s with the non-primary motor areas, the basal ganglia, and the motor nuclei of the thalamus.

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Keywords: Repetitive transcranial magnetic stimulation; Positron emission tomography; Effective connectivity; Plasticity; Stroke; Constraint-induced movement therapy

Introduction

Neuroimaging has provided a wealth of knowledge about brain areas underlying stroke recovery (e.g., Chollet et al., 1991; Weiller et al., 1992, 1993; Cramer et al., 1997; Dettmers et al., 1997; Honda et al., 1997; Cao et al., 1998; Seitz et al., 1998; Nelles et al., 2001; Ward et al., 2003a,b). Yet, we still do not understand how these areas interact with each other. This is because current methods have a limited ability to discern how different brain regions influence each other. A number of studies have examined connectivity using correlation-based analyses to determine similarities in regional variations of task-related changes in cerebral activity (McIntosh and Gonzalez-Lima, 1994; Friston, 1994; Friston et al., 1996). Although these approaches provide valuable information about connectivity in a behavioral context, co-activations may reflect relationships between different task components rather than true effective connectivity.

We used a perturb-and-measure approach in which transcranial magnetic stimulation (TMS) was combined with positron emission tomography (PET) to examine effective connectivity of M1 (for a review, see Paus, 2005). The advantage of this approach is that it serves as a behavior-independent assay of connectivity between different task components rather than true effective connectivity.

We used a perturb-and-measure approach in which transcranial magnetic stimulation (TMS) was combined with positron emission tomography (PET) to examine effective connectivity of M1 (for a review, see Paus, 2005). The advantage of this approach is that it serves as a behavior-independent assay of connectivity between different task components rather than true effective connectivity.

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proposed that the trains of stimulation resulted in an activation of local inhibitory mechanisms and a subsequent reduction of excitatory synaptic activity in the stimulated region and in the interconnected network.

We applied a similar protocol to examine changes in M1 and its effective connectivity in stroke patients who underwent 3 weeks of constraint-induced movement therapy (CI therapy; Taub et al., 1993). Improvements on various motor tests were observed after therapy. During the TMS/PET sessions, we applied trains of subthreshold 10-Hz repetitive TMS over the probabilistic hand representation of the ipsilesional and contralesional M1s and varied the number of TMS trains delivered during each scan. This stimulation protocol allowed us to examine changes in the local response of the ipsilesional and contralesional M1 as well as changes in their effective connectivity with interconnected structures in the brain.

**Methods**

**Patients**

One female and six male patients participated in the study (49–78 years of age, mean ± SEM, 65.9 ± 1.4 years of age). Table 1 lists their sex, age, full-scale IQ rating, months after stroke, type of stroke, side of paresis, and the location of their lesions at the time of the study as determined with magnetic resonance imaging [MRI; 176–192 contiguous 1-mm-thick T1-weighted sagittal slices; Siemens (Erlangen, Germany) Vision 1.5-T system]. Six out of the seven patients had a strong right-hand preference before their stroke as determined by a handedness questionnaire (Crovitz and Zener, 1965). The one patient with a left-hand preference before stroke suffered an infarct to the right side of his brain, which left him with a paresis of his dominant hand (Patient 1). All patients had received conventional physiotherapy after their stroke and participated in the study more than one year after their stroke. The Institutional Review Board of McGill University and the Research Ethics Board of the Montreal Neurological Institute and Hospital approved all procedures. Patients gave informed consent in accordance with the Declaration of Helsinki.

We identified potential subjects from a database at the McGill University Health Centre. We first screened these patients by a telephone interview and then invited them to meet with us to discuss the project. In the face-to-face interview, we verified whether they met a minimum motor criterion of being able to extend their wrist against gravity and to make a fist and extend their fingers apart against gravity. We did not include candidates who could not make these basic hand movements, reported having problems with pain in association with arm and hand movements, and/or had a personal or family history of epilepsy. A neuropsychologist at the Montreal Neurological Hospital examined each candidate to verify that she or he did not have any serious uncontrolled medical problems. Having completed their neurological examination, the neuropsychologist involved in this study (G Leonard) evaluated the candidates’ cognitive status; IQ scores are provided in Table 1. The assessment included the Wechsler Abbreviated Scale of Intelligence, the Wechsler Memory Scale-III, Problem Solving (Tower of London), Trails A and B, Word Fluency, and Cancellation. Review of the results enabled us to determine whether the candidates’ language skills were sufficient to understand task instructions and provide informed consent. Candidates then underwent MRI so that we could examine the extent of their lesions. We excluded candidates with brainstem lesions or lesions that extended into the probabilistic hand representation of M1.

**Movement therapy**

Patients underwent a rehabilitation program based on CI therapy (Taub et al., 1993, 2002). The therapy consisted of two components: (1) restricting movement of the unaffected arm, and (2) training the affected arm by a procedure called shaping. Patients were given a padded safety mitt that enclosed the fingers and the wrist. Each patient agreed to wear this restraint on their unaffected arm for 90% of waking hours. A home treatment contract was made with the patient outlining the agreed-upon activities in which the patient could and could not participate while wearing the mitt. This contract served to encourage patients to wear the restraint except in situations where safety might be compromised. During the three-week period, all patients, except for one patient, spent four hours at the laboratory where they performed shaping exercises under our supervision for 14 days. For the one patient who did not come to the laboratory (Patient 2), we had agreed instead to come to her apartment for the same amount of time to supervise her shaping exercises. Shaping refers to the practice of the affected arm in which a given movement is approached in small steps of progressively increasing difficulty (Taub et al., 1994). Patients performed a total of 10–12 different shaping exercises, of which they performed 5–6 a day. Each shaping exercise was tailored to the deficit of the patient and targeted movements we thought had the most potential to improve. We administered

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>IQ</th>
<th>Months after stroke</th>
<th>Type of stroke</th>
<th>Side of paresis</th>
<th>Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>68</td>
<td>113</td>
<td>34</td>
<td>Ischemic</td>
<td>L</td>
<td>PMd, medullary substance Putamen, internal capsule</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>78</td>
<td>89</td>
<td>51</td>
<td>Ischemic</td>
<td>R</td>
<td>Frontal–parietal white matter</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>49</td>
<td>84</td>
<td>12</td>
<td>Ischemic</td>
<td>R</td>
<td>Internal capsule</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>54</td>
<td>74</td>
<td>15</td>
<td>Hemorrhage</td>
<td>R</td>
<td>Internal capsule</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>70</td>
<td>88</td>
<td>12</td>
<td>Ischemic</td>
<td>L</td>
<td>MSI, WM hypodensity</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>73</td>
<td>76</td>
<td>16</td>
<td>Ischemic</td>
<td>R</td>
<td>Internal capsule, VL thalamus</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>69</td>
<td>127</td>
<td>21</td>
<td>Hemorrhage</td>
<td>R</td>
<td>Internal capsule, VPL thalamus</td>
</tr>
</tbody>
</table>

Mean: 65 93 23

Abbreviations: PMd = dorsal premotor cortex, MSI = multiple subcortical infarcts, WM = white matter.
motor tests before (pre), immediately after (post), and 1 month after therapy (follow-up).

Motor tests

We assessed functional ability of the affected arm with the Wolf Motor Function Test (WMFT; Wolf et al., 1989, Taub et al., 1993) in which patients performed 13 timed tasks in front of a video camera. The WMFT provided scores on a scale from zero to five, with zero indicating that the patient could not perform the task and five indicating normal use. The Actual Amount of Use Test (AAUT; Taub et al., 1998) addressed the issue of amount of use of the affected arm when a task is not requested. We filmed performance on the AAUT in a setting where patients were unaware that they were being tested by prompting them to perform predetermined activities. The AAUT provided scores on a scale from zero to two, with zero indicating that the patient did not attempt to use the affected arm and two indicating that the patient used the affected arm and that its use was functional and not rudimentary. An undergraduate student rated the WMFT and AAUT while being unaware when testing occurred. Before the student rated any of our videos, she spent a considerable amount of time under our supervision learning how to score training videos that we obtained from the CI therapy group at the University of Alabama at Birmingham (Birmingham, AL, USA). She only began rating our videos once we determined that she could score the training videos consistently with the scores supplied along with the training videos.

We attempted to assess real-world outcome with the motor activity log (MAL; Taub et al., 1993), which consisted of an interview with the patient designed to address transfer of acquired skills from the laboratory to the home setting. It provided scores on a scale from zero to five for both the quality of movement and the amount of use of the affected arm in specific tasks commonly carried out at home. Zero indicated the lowest possible scores and five indicated the highest possible scores. We administered additional tests to assess coordination, speed, and strength in both arms. Patients used a hand-held stylus to tap on a board that had two circular brass plates, each plate divided into four distinct pie-shaped sectors numbered 1, 2, 3, and 4, respectively (Thurstone, 1944). Using this apparatus, we tested the patients’ ability to perform simple tapping on one sector and spatially ordered sequential tapping on all four sectors (Leonard et al., 1988). We also measured pinch and grip strength using dynamometers.

Overview of brain-mapping procedures

We designed the TMS/PET protocol to enable us to compare the effects of stimulating the ipsilesional and contralesional M1s at the pre and post sessions (Fig. 1). We stimulated over the probabilistic hand representation of M1 in each of the two hemispheres and measured the cerebral blood-flow (CBF) response with PET. We acquired a total of seven 60-s scans in each of the two sessions using the $^{15}$O-labeled H$_2$O bolus method (Raichle et al., 1983). During the first, second, and third scans, we delivered a different number of TMS trains over M1 in one hemisphere (5, 15, and 30 trains of 10-Hz stimulation, each train with a duration of 1 s). The fourth scan served as a baseline scan without TMS. During the fifth, sixth, and seventh scans, we stimulated over the opposite M1 using the same stimulation parameters as in the first three scans. We applied the same stimulation intensity when stimulating over both the ipsilesional and contralesional M1s and during both the pre and post sessions. In a given subject, we counter-balanced the orders of the number of TMS trains delivered and the sites of stimulation. We also acquired 10-min transmission scans after we positioned the TMS coil over the first and second target sites.

Fig. 1. TMS/PET Protocol. Patients underwent TMS/PET before and after therapy. During the first three water-bolus scans, we delivered a different number of TMS trains over one of the two M1s; that is, 5, 15, and 30 trains of 1-s 10-Hz stimulation. During the last three water-bolus scans, we stimulated over the other M1 using the same stimulation as in the previous water-bolus scans. Stimulation intensity was the same for both M1s as well as for before and after therapy.
Positron emission tomography

We instructed subjects to keep their eyes closed during scanning. We measured CBF with a CTI/Siemens HR+63-slice tomograph scanner operated in 3-D acquisition mode. For each scan, we injected 8.5 mCi of $^{15}$O-labeled H$_2$O into the antecubital vein of the unaffected arm. Acquired CBF images were smoothed with a 14-mm Hanning filter, normalized for differences in global CBF (‘normalized CBF’), co-registered with individual MR images (Woods et al., 1993), and transformed into standardized stereotactic space (Talairach and Tournoux, 1988) by means of an automated feature-matching algorithm (Collins et al., 1994) using the average 305 MNI brain as a template. We flipped CBF images in standardized space in Patients 1 and 5 so that the left hemisphere represented the affected hemisphere in all patients. The flipping of these images in standardized stereotactic space was deemed appropriate given the spatial resolution of PET acquisition (2.5 mm slices). We placed four 0.5-mm thick sheets of well-grounded mu-metal in the scanner gantry to protect the photomultipliers inside the scanner from the effects of the coil-generated magnetic field. The mu-metal, however, attenuates gamma rays and in turn decreases the number of detected coincidence counts (Paus, 2002). We used the transmission scans to correct for the attenuation of gamma rays caused by all objects in the scanner, including the coil, the coil mount, and the metal sheets.

Transcranial magnetic stimulation

Before each PET session, we determined resting motor thresholds (rMT) by varying the stimulation intensity until a level was reached at which observable hand muscle twitches were reliably induced for at least 50% of stimulations, and lesser levels of stimulation failed to induce consistently these same contractions (Table 2). During scanning, we carried out TMS using a Cadwell high-speed magnetic stimulator (Cadwell Inc., Kennewick, Washington, USA) and a Cadwell circular stimulating coil with a cooling system mounted over its outer casing (external diameter, 9 cm). We applied 1-s trains of 10 Hz at a subthreshold intensity at 95% rMT of either the affected or unaffected arm, whichever was lowest at the pre session (Table 2). In Patients 4 and 6, we could not detect rMTs for one hand and therefore set the stimulation intensity at 95% rMT of the opposite hand. To verify that we stimulated below the rMT, we used electromyography to record activity from the first dorsal interosseous muscles. TMS applied during scanning did not induce any overt movements or any significant electromyographic responses.

We used a five-step procedure for coil positioning (Paus et al., 1997). First, we transformed the subject’s M1 into standardized space (Talairach and Tournoux, 1988; Collins et al., 1994). Second, we derived a probabilistic location for M1 ($X = \pm31$, $Y = -22$, $Z = 52$; Paus et al., 1998) using information gained in previous studies that measured cerebral activity during volitional hand movements. Third, we transformed this probabilistic location to the subject’s brain coordinate space for both hemispheres. Fourth, we used frameless stereotaxy to mark with a felt pen the location on the scalp that was closest to the target sites in each of the two hemispheres marked on the subject’s MRI (Brainsight software: Rogue Research Inc., Montreal, Quebec, Canada; Polaris System: Northern Digital Inc., Waterloo, Ontario, Canada). The final step required us to position the TMS coil over the marked locations on the scalp. With the patient lying on the bed of the scanner, we inserted a bite-bar in their mouth, placed them inside the scanner, positioned the coil over one of the marked locations on the scalp, and then used a mechanical arm to lock in place the coil. We positioned the coil so that its lateral rim was placed over the target location with the rest of the coil tilted away from the skull. The anterior tip of the coil was positioned in the anterior direction and the handle of the coil was parallel to the interhemispheric fissure and pointed backwards.

Verification of coil positions

Using a procedure described in detail elsewhere (Paus and Wolfarth, 1998), we used the transmission scans to verify final coil positioning relative to the acquired PET and MR images (Fig. 2). These images showed us the coil’s position relative to the subject’s head. We projected virtual rods perpendicular to the plane of the coil from where the coil touched the scalp. Following PET-to-PET, PET-to-MRI, and MRI-to-standardized space transformations, we superimposed the location of these virtual rods on an average anatomical MRI of all patients. This end product served to verify whether the TMS coil targeted our intended sites of stimulation (Fig. 2). Overall, the projected coil trajectories passed through the ‘hand knob’ of M1 as defined anatomically by Yousry et al. (1997) and showed similar consistency as in our previous TMS/PET studies (Chouinard et al., 2003; Barrett et al., 2004).

Analyses of motor performance

For tests in which we obtained measures of performance for both the affected and unaffected arms (i.e., simple tapping, sequential tapping, pinch strength, and grip strength), we provide performance ratios between the affected and unaffected arms as well as mean scores for both arms. For all tests, we

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre session</th>
<th>Post session</th>
<th>TMS intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ipsilesional</td>
<td>Contralesional</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>M1</td>
<td>Ipsilesional</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M1</td>
</tr>
<tr>
<td>1</td>
<td>85</td>
<td>70</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>71</td>
<td>64</td>
<td>66</td>
</tr>
<tr>
<td>4</td>
<td>Absent</td>
<td>69</td>
<td>Absent</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>45</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>66</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>7</td>
<td>54</td>
<td>54</td>
<td>54</td>
</tr>
</tbody>
</table>

Values represent percentages of the maximum output of the stimulator.
evaluated the effects of therapy using repeated-measures ANOVA using session (pre, post, and follow-up) as a within-subject factor and Tukey’s HSD tests, which corrected for multiple comparisons, for all post-hoc pairwise comparisons.

To reduce the number of factors used to later correlate improvement with post−pre CBF differences, we first calculated for each test the percentage change in performance at the post session \[\left(\left((\text{post}/\text{pre}) - 1\right) \times 100\right)\], and then determined relationships between these percentages with a principle-components factor-analysis using Varimax rotation in SPSS (Statistical Package for the Social Sciences; Chicago, IL, USA). We also report effect sizes (\(\varepsilon\)) for the post and follow up sessions; these were calculated by subtracting the post and follow up means from the mean of the pre session and then dividing this difference by the standard deviation of the post-treatment session (Glass et al., 1981).

**Analyses of cerebral blood-flow**

We performed two statistical analyses of the CBF data using in-house software of the MNI (http://www.bic.mni.mcgill.ca/users/sylvain/doc/html/dot/index.html). For the first analysis, we examined whether the relationship between CBF and the number of TMS trains delivered over M1 differed at the post session compared with the pre session (i.e., their Session × TMS Trains interaction). We used ANOVA to test whether at a given voxel an interaction between session (pre and post) and TMS trains (5, 15, and 30 trains) was significant after having removed the effects of subjects.

For the second analysis, we used a four-step procedure to examine relationships between improvement and post−pre CBF differences (i.e., their linear regression). First, we calculated the average CBF response to TMS (i.e., the three TMS train conditions pooled). Second, we subtracted CBF acquired during the base scan from the average CBF response to TMS (average − base). Third, we calculated the difference of these subtractions at the pre session from the post session \[\left(\text{average} - \text{base}\right)_{\text{post}} - \left(\text{average} - \text{base}\right)_{\text{pre}}\].

The fourth step tested whether at a given voxel the slope of the linear regression between improvement \[\left(\left((\text{post}/\text{pre}) - 1\right) \times 100\right)\] and these CBF differences were significantly different from zero. We generated \(T\)-statistical maps by calculating \(T\)-values at each voxel constituting the entire brain volume scanned. After generating these maps, we superimposed the resulting images with the transformed averaged MRI of all subjects in standardized space (Talairach and Tournoux, 1988). We performed exploratory searches of the entire brain with correction for multiple comparisons based on random-field theory (Worsley et al., 1992). For this type of search, we considered values equal to or exceeding a criterion of \(T = 4.2\) as significant \((P_{\text{uncorr}} < 0.000002, \text{two-tailed})\), yielding a false-positive rate of \(P_{\text{corr}} \leq 0.05\) in 218 resolution elements (each of which has dimensions 14 × 14 × 14 mm) for a gray-matter volume of 600cm³. Exploratory searches did not reveal any significant changes.

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**Fig. 2. Projected coil trajectories.** The 10-min transmission scans acquired during the PET sessions allowed us to see the coil’s position relative to the subject’s head. We projected virtual rods perpendicular to the plane of the coil from where the coil touched the scalp. Following a series of transformations, we superimposed the location of these virtual rods on an average MRI. The figure illustrates the projected coil trajectories (red circles) derived from the TMS coil placed over the ipsilesional M1 (top left) and contralesional M1 (top right); these coil trajectories served to verify whether the TMS coil targeted our intended sites of stimulation. Crosses represent the intended sites of stimulation. Overall, the projected coil trajectories passed through the ‘hand knob’ of M1 as defined anatomically by Yousry et al. (1997) and showed similar consistency as in our previous TMS/PET studies (Chouinard et al., 2003; Barrett et al., 2004).
In one of our previous TMS/PET studies (Paus et al., 1998), we demonstrated in healthy volunteers that a similar protocol as the one used for this study reduces local CBF as a function of the number of TMS trains delivered over M1. We therefore performed one-tailed directed searches at our sites of stimulation with correction for multiple comparisons based on random-field theory (Worsley et al., 1992). We considered values equal to or exceeding a criterion of $T = 2.7$ as significant ($P_{\text{uncorr}} < 0.002$, one-tailed), yielding a false-positive rate of $P_{\text{corr}} \leq 0.05$ in two resolution elements (each of which has dimensions of $14 \times 14 \times 14 \text{ mm}^3$) for a tissue volume of $5 \text{ cm}^3$.

We also performed two-tailed directed searches in brain regions known to be connected to M1 (primary somatosensory area, dorsal and ventral premotor areas, supplementary motor area, cingulate motor areas, and basal ganglia) with correction for multiple comparisons based on random-field theory (Worsley et al., 1992). We considered values equal to or exceeding a criterion of $T = 3.0$ as significant ($P_{\text{uncorr}} < 0.002$, two-tailed), yielding a false-positive rate of $P_{\text{corr}} \leq 0.05$ in two resolution elements (each of which has dimensions of $14 \times 14 \times 14 \text{ mm}$) for a tissue volume of $5 \text{ cm}^3$. To perform this search, we relied on both anatomical studies performed by others in the monkey and previous neuroimaging studies that mapped their putative homologues. We provide a detailed description of how these regions of interest were formulated in one of our previous TMS/PET studies that used the same directed search to establish distal brain regions influenced by repetitive TMS applied over M1 in healthy volunteers (Chouinard et al., 2003).

Results

Motor tests

Table 3 lists correlations among the different tests included in the principle-components factor-analysis and Table 4 summarizes the results from the principle-components factor-analysis. The analysis revealed that the percentage change in performance at the post session for the WMFT, the AUTT, the sequential tapping, and the grip strength could be expressed as a single factor (Component 1). We will refer to this component (1) later as improvement on the non-self-report tests. The analysis also revealed that the percentage change in performance at the post session for both the quality of movement and amount of use scores obtained in the MAL could be expressed as a second factor (Component 2), which could relate to the fact that these scores were the only self-report measures used in this study. The analysis also revealed that the percentage change in performance at the post session for simple tapping could be expressed as a third factor (Component 3). In this test, patients did not demonstrate improvements.

Patients improved on the WMFT, the AAUT, the sequential tapping, and the grip strength (Table 5). For the WMFT, patients had an initial mean score of 3.5 for functional ability, which lies between a score for movements influenced by synergy and/or made with effort (3), and a score for movements that were not quite as fast or accurate as normal (4). Patients improved after therapy with mean scores of 3.9 at both the post session ($P < 0.01$) and follow-up session ($P < 0.01$). For the AUTT, patients had an initial mean score of 1.4 for amount of use, which lies between a score for rudimentary use (1) and a score for functional use (2). Patients demonstrated improvements at the post session with a mean score of 1.7 ($P < 0.01$), but this change in performance was not maintained at follow-up. A significant decrease in the amount of use was observed at the follow-up session compared with the post session ($P < 0.05$). Patients performed better on sequential tapping at the post session compared with the pre session ($P < 0.05$). Also, patients had stronger grip strength at the post session compared with the pre session ($P < 0.01$), but not at follow-up. A significant decrease in grip strength was observed at the follow-up session compared with the post session ($P < 0.05$).

For the MAL, patients reported improvements for the quality of movement and the amount of use of the affected arm in specific tasks commonly carried out at home (Table 5). At the pre session, patients reported mean scores of 2.6 for both quality of movement and amount of use. For quality of movement, this lies between a score for ‘poor’ (2) and a score for ‘fair’ (3) and for amount of use, this lies between a score for ‘rarely’ (2) and a score for ‘1/2 as much as before stroke’ (3). After therapy, patients reported improvements in quality of movement with mean scores of 3.9, which is close to a score for ‘almost normal’ (4), at both the post session ($P < 0.01$) and follow-up session ($P < 0.01$). Patients also reported improvements in amount of use with mean scores of 3.9, which is close to a score for ‘3/4 as much as before stroke’ (4), at both the post session ($P < 0.01$) and follow-up session ($P < 0.01$).

Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Wolfe motor function test</td>
<td>1.00</td>
<td>0.83*</td>
<td>0.53</td>
<td>0.53</td>
<td>0.53</td>
<td>0.53</td>
<td>0.53</td>
</tr>
<tr>
<td>2. Actual amount of use test</td>
<td>0.83*</td>
<td>1.00</td>
<td>0.53</td>
<td>0.53</td>
<td>0.53</td>
<td>0.53</td>
<td>0.53</td>
</tr>
<tr>
<td>3. Unimanual sequential tapping, AA/UA ratio</td>
<td>0.53</td>
<td>0.53</td>
<td>1.00</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>4. Grip strength, AA/UA ratio</td>
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<td>0.53</td>
<td>0.31</td>
<td>1.00</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
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<tr>
<td>5. Motor activity log, quality of movement</td>
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<td>0.53</td>
<td>0.31</td>
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<td>1.00</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>6. Motor activity log, amount of use</td>
<td>0.53</td>
<td>0.53</td>
<td>0.31</td>
<td>0.18</td>
<td>0.18</td>
<td>1.00</td>
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</tr>
<tr>
<td>7. Unimanual simple tapping, AA/UA ratio</td>
<td>0.53</td>
<td>0.53</td>
<td>0.31</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>1.00</td>
</tr>
<tr>
<td>8. Pinch strength, AA/UA ratio</td>
<td>0.53</td>
<td>0.53</td>
<td>0.31</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Values represent correlations ($r$) between the percentage change in performance at the post session $[(\text{post/pre} - 1) \times 100]$ measured in the different tests. Asterisks (*) denote significant correlations ($df = 5, P < 0.05$). Abbreviations: AA = affected arm, UA = unaffected arm.
Table 4  
Results obtained from the principle-components factor-analysis

<table>
<thead>
<tr>
<th>Test</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>r</td>
</tr>
<tr>
<td>Actual amount of use test</td>
<td>0.88*</td>
</tr>
<tr>
<td>Unimanual sequential tapping, AA/UA ratio</td>
<td>0.82*</td>
</tr>
<tr>
<td>Motor activity log, quality of movement</td>
<td>0.08</td>
</tr>
<tr>
<td>Motor activity log, amount of use</td>
<td>0.22</td>
</tr>
<tr>
<td>Unimanual simple tapping, AA/UA ratio</td>
<td>-0.07</td>
</tr>
<tr>
<td>Pinch strength, AA/UA ratio</td>
<td>-0.11</td>
</tr>
</tbody>
</table>

The table represents the Varimax-rotated matrix derived from the principle-components factor-analysis performed in SPSS. Values represent correlations (r) between the percentage change in performance at the post session [(post/ pre) − 1] × 100] for each test and each component representing a partition of the overall variance. Asterisks (*) denote significant correlations (df = 5; P < 0.05). Abbreviations: AA = affected arm, UA = unaffected arm.

Cerebral blood-flow

We examined whether the relationship between the number of TMS trains delivered and CBF differed at the post session compared with the pre session (i.e., their Session × TMS Trains interaction). Fig. 3 summarizes the results obtained from this analysis. When TMS was applied over the ipsilesional M1, the effects of TMS trains differed between the two sessions locally in the ipsilesional SM1 (T = −2.8; one-tailed directed search). The distal effects of TMS trains differed almost significantly between the two sessions in the contralesional M1 (T = 2.9) and significantly in the ipsilesional cingulate motor area (T = −3.5). To understand these Session × TMS Trains interactions, we extracted CBF values at the X, Y, and Z coordinates of the three peaks and performed ANOVA. After therapy, the number of TMS trains delivered co-varied negatively with local CBF in the ipsilesional SM1 (P one-tailed < 0.05), positively with distal CBF in the contralesional M1 (P two-tailed < 0.005), and negatively with distal CBF in the ipsilesional cingulate motor area (P two-tailed < 0.01). CBF data acquired during stimulation over the contralesional M1 did not reveal any changes.

We also assessed the relationship between improvement and post–pre CBF differences (i.e., their linear regression). Fig. 4 demonstrates brain areas in which the slope of the linear regression between improvement on the non-self-report tests (Component 1) and CBF differences differed significantly from zero. When TMS was applied over the ipsilesional M1, improvement co-varied negatively with local CBF differences in the ipsilesional M1 (T = −2.9, one-tailed directed search) and distal CBF differences in the contralesional VL thalamus (T = −3.6). In contrast, improvement on the MAL (Component 2) did not co-vary with CBF differences anywhere in the brain. When TMS was applied over the contralesional M1, improvement on the non-self-report tests co-varied negatively with local CBF differences in the contralesional primary sensorimotor area (T = −3.1) and co-varied positively with distal CBF differences in the contralesional globus pallidus (T = 4.1) and ipsilesional putamen (T = 3.5). Improvement on the MAL co-varied positively with distal CBF differences in the contralesional VPL thalamus (X = 21, Y = −19, Z = 10; T = 3.4) and contralesional ventral premotor area in the precentral operculum (X = 62, Y = 0, Z = 12; T = 3.2).

Discussion

The behavioral data confirm previous reports that improvements in coordination and activities of daily living can occur

Table 5  
Motor performance on various tests before (pre), immediately after (post), and 1 month after (follow-up) therapy

<table>
<thead>
<tr>
<th>Test</th>
<th>Pre mean ± SD</th>
<th>Post mean ± SD</th>
<th>Follow-up mean ± SD</th>
<th>F(2,12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ε</td>
<td>ε</td>
<td>ε</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual amount of use test</td>
<td>3.50 ± 0.69</td>
<td>3.91 ± 0.66*</td>
<td>0.56</td>
<td>3.90 ± 0.57*</td>
<td>0.62</td>
</tr>
<tr>
<td>Unimanual sequential tapping, AA/UA ratio</td>
<td>0.74 ± 0.24</td>
<td>0.85 ± 0.20*</td>
<td>0.54</td>
<td>0.83 ± 0.20</td>
<td>1.20</td>
</tr>
<tr>
<td>Grip strength, AA/UA ratio</td>
<td>0.58 ± 0.30</td>
<td>0.70 ± 0.29*</td>
<td>0.44</td>
<td>0.59 ± 0.29*</td>
<td>0.60</td>
</tr>
<tr>
<td>Motor activity log, amount of use</td>
<td>2.59 ± 1.06</td>
<td>3.91 ± 0.74*</td>
<td>1.04</td>
<td>3.88 ± 0.94*</td>
<td>1.10</td>
</tr>
<tr>
<td>Unimanual simple tapping, (AA)/UA ratio</td>
<td>0.77 ± 0.28</td>
<td>0.79 ± 0.27</td>
<td>0.70</td>
<td>0.71 ± 0.30</td>
<td>1.27</td>
</tr>
<tr>
<td>Pinch strength, (AA)/UA ratio</td>
<td>0.82 ± 0.33</td>
<td>0.91 ± 0.47</td>
<td>0.88</td>
<td>0.88 ± 0.38</td>
<td>1.28</td>
</tr>
<tr>
<td>Unimanual sequential tapping, AA (tap/s)</td>
<td>2.17 ± 1.20</td>
<td>2.64 ± 1.56*</td>
<td>0.30</td>
<td>2.56 ± 1.35*</td>
<td>0.38</td>
</tr>
<tr>
<td>Grip strength, affected arm (lb)</td>
<td>3.41 ± 23.4</td>
<td>39.8 ± 23.4</td>
<td>0.25</td>
<td>35.4 ± 23.1*</td>
<td>0.25</td>
</tr>
<tr>
<td>Grip strength, unaffected arm (lb)</td>
<td>58.0 ± 20.2</td>
<td>35.9 ± 18.1</td>
<td>0.74</td>
<td>58.3 ± 19.2</td>
<td>0.74</td>
</tr>
<tr>
<td>Unimanual simple tapping, AA (tap/s)</td>
<td>4.39 ± 1.42</td>
<td>4.69 ± 1.62</td>
<td>0.22</td>
<td>4.29 ± 1.76</td>
<td>0.22</td>
</tr>
<tr>
<td>Pinch strength, affected arm (lb)</td>
<td>13.5 ± 4.1</td>
<td>13.8 ± 5.6</td>
<td>0.05</td>
<td>13.8 ± 2.8</td>
<td>0.05</td>
</tr>
<tr>
<td>Pinch strength, unaffected arm (lb)</td>
<td>18.8 ± 8.5</td>
<td>17.6 ± 7.0</td>
<td>17.8 ± 6.8</td>
<td>1.29</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Asterisks (*) denote improved performance at the post session and follow-up compared with the pre session (P < 0.05). Daggers (†) denote improvements at the post-session that were not maintained at follow-up (P < 0.05). We also report effect sizes (ε) for the post session and follow-up; these were calculated by subtracting the post and follow-up means from the mean of the pre session and then dividing this difference by the standard deviation of the post-treatment session (Glass et al., 1981). Abbreviations: AA = affected arm, UA = unaffected arm.
with CI therapy even when the therapy is conducted one or more years after stroke (Taub et al., 1993; Miltner et al., 1999). The novelty of this study is that we demonstrate changes in the local CBF response of TMS in the ipsilesional and contralesional M1, changes in the strength of interhemispheric connectivity between M1s, and changes in the effective connectivity of the ipsilesional and contralesional M1s with the non-primary motor areas, the basal ganglia, and the motor nuclei of the thalamus.

**Motor improvements**

The side of hemiparesis would seem important for determining the effect of movement therapy, with patients having greater motivation to regain use of a premorbid dominant arm rather than a premorbid non-dominant arm. This was not the case in Patient 4 who was the only participant that received therapy for a premorbid non-dominant arm. His percent change in performance was always in the top three patients for all motor tests for which we report improvements. Miltner et al. (1999) demonstrated in a larger sample of patients, all with right-arm dominance before stroke, that patients with a left-sided hemiparesis exerted as large a treatment effect after CI therapy as those with a right-sided hemiparesis.

The effect sizes reported for the WMFT and the MAL (Table 5) are consistent with previous studies that examined the effects of CI therapy on motor function after stroke (Taub et al., 1993; Miltner et al., 1999). The patients, however, did not maintain improvements in the amount of use of the affected arm as measured with the AAUT. It is difficult to determine why this would be the case considering that patients reported with the MAL that they maintained improvements in the amount of use of the affected arm at home. We also demonstrate improvements with smaller effect sizes in sequential tapping and grip strength (Table 5). No improvements were observed in the affected arm for simple tapping and pinch strength. Successful performance on these latter tests may depend more on the integrity of the corticospinal tract and in turn may not allow the development of compensatory skills to enhance performance (Nakayama et al., 1994). Corticospinal fibers with a direct influence on spinal motor-neurons are important for pinch strength and have less of a role during the co-contraction of a number of muscles used in the hand grip (Muir and Lemon, 1983).

**Issues related to brain-mapping procedures**

We acquired CBF while concurrently applying TMS over M1 during a resting state. This approach differs from some TMS/PET studies that acquire CBF during volitional hand movements performed by healthy volunteers after a period of repetitive TMS (Lee et al., 2003; Siebner et al., 2003). Although these TMS/PET studies...
PET studies provide valuable information about connectivity during a behavioral context, acquired co-activations may reflect relationships between different task components rather than true effective connectivity. In contrast, we applied TMS during a resting state to allow for a more direct assessment of connectivity between M1 and other structures in the brain. Bestmann et al. (2003, 2005) have also used this approach during functional MRI in healthy volunteers.

We did not define the site of stimulation for M1 by determining the ‘hot spot’ where stimulation results in the maximum response of a contralateral hand muscle. This location can shift medially in some patients and laterally in others after rehabilitative therapy (Liepert et al., 1998). To enable more appropriate comparisons of TMS-induced changes in CBF between sessions, we decided instead to stimulate consistently in the same location for all patients before and after therapy using a circular coil that stimulates less focally than a figure-of-eight coil. We defined the sites of stimulation for M1 based on previous studies that measured cerebral activity during volitional hand movements performed by healthy volunteers (Paus et al., 1998). The transmission scans acquired during the TMS/PET sessions allowed us to verify whether the TMS coil targeted the intended sites of stimulation (Fig. 2). These trajectories passed consistently through the ‘hand knob’ of M1 as defined anatomically by Yousry et al. (1997). We should mention that although these trajectories demonstrate that we positioned the TMS coil properly to target M1, repetitive TMS using a circular coil...
might have also affected surrounding tissue in the primary somatosensory and dorsal premotor cortices.

Changes in the local response of the ipsilesional M1

Paus et al. (1998) demonstrated in healthy volunteers that the local CBF response to subthreshold 10-Hz repetitive TMS over M1 decreases with the number of TMS trains delivered during each scan. The trains of TMS may have resulted in a preferential activation of local inhibitory circuits and a subsequent reduction of excitatory synaptic activity in the stimulated region. This interpretation is supported by the following evidence. First, the pharmacological administration of γ-aminobutyric acid (GABA) agonists in humans can enhance intra-cortical inhibition in M1 (Ziemann et al., 1996) and decrease CBF in distinct brain regions when used in combination with PET to determine hemispheric dominance (Roland and Friberg, 1988). Second, four 10-pulse trains of repetitive TMS at frequencies from 2 to 15 Hz can prolong the duration of the silent period without changing corticospinal excitability (Romeo et al., 2000).

When TMS was applied over the ipsilesional M1, both the number of TMS trains delivered and improvement on the non-self-report tests co-varied negatively with local CBF differences. These findings may reflect a post-therapy strengthening of local inhibitory neurons. These neuronal pools are important for the fractionation of movements between different distal and proximal muscles (Keller, 1993). The blockade of GABAergic inhibition in the macaque monkey’s M1 disrupts the spatiotemporal sequence of movement patterns performed by the forelimb (Matsumura et al., 1991). Interestingly, the local CBF response as a function of the number of TMS trains delivered differed between the two sessions at a site (Fig. 3) more lateral to the intended site of stimulation (Fig. 2). This may reflect a possible reorganization of M1. This displacement fits well with previous studies that demonstrate shifts in the hand representation of M1 after rehabilitation of the forelimb following stroke in the squirrel monkey (Nudo et al., 1996), rehabilitation of the arm following stroke in the human (Liepert et al., 1998, 2000), and a period of recovery after stroke in the human (Weiller et al., 1993).

The lack of an increase in local CBF response may reflect both the stimulation intensity and the low number of pulses delivered during scanning (50, 150, and 300 pulses/scan). A small number of pulses of high-frequency (≥5 Hz) repetitive TMS can increase corticospinal excitability only when applied at suprathreshold intensities (Pascual-Leone et al., 1994; Wu et al., 2000). At subthreshold intensities, a greater number of TMS pulses must be delivered for high-frequency repetitive TMS to increase corticospinal excitability effectively. Maeda et al. (2000) demonstrated that 10-Hz repetitive TMS applied at 90% of the resting motor threshold can increase corticospinal excitability after 1600 pulses and not after 240 pulses. Quatrarone et al. (2005) demonstrated similarly that 5-Hz repetitive TMS applied at 90% resting motor threshold can increase corticospinal excitability after 900, 1200, and 1500 pulses and not after 300 and 600 pulses.

Changes in the local response of the contralesional M1

When TMS was applied over the contralesional M1, improvement on the non-self-report tests co-varied negatively with local CBF differences. The role of the contralesional M1 during recovery remains unclear. M1 has no direct access to the spinal motor-neurons that innervate distal arm muscles on the same side of the body (Liu and Chambers, 1964; Ralston and Ralston, 1985). This observation rules out the possibility that uncrossed corticospinal fibers from the contralesional M1 could provide a substrate for substitution. The contralesional M1 does nonetheless undergo adaptive changes. Patients who recover well from one stroke and have a second stroke in the opposite hemisphere will not only have a new contralateral hemiparesis, but will also have a reappearance of the original deficits caused by the first stroke (Fisher, 1992; Lee and van Donkelaar, 1995). Similarly, a recent study demonstrates that repetitive TMS applied over the contralesional M1 in stroke patients with small capsular infarcts disrupts more their ability to perform sequential finger movements >8 months after stroke when they regained complete use of their affected arm compared with healthy control subjects (Lotze et al., 2005). A number of neuroimaging studies also demonstrate increased activity in the contralesional M1 as recovered patients perform simple hand movements (Chollet et al., 1991; Weiller et al., 1993; Cramer et al., 1997; Honda et al., 1997; Cao et al., 1998). The increased activity in the contralesional M1 appears to emerge consistently with co-activity in the ipsilesional M1 provided that damage in the ipsilesional M1 is limited (Chollet et al., 1991; Weiller et al., 1993; Honda et al., 1997; Cao et al., 1998).

Changes in interhemispheric connectivity between M1s

When TMS was applied over the ipsilesional M1, the effects of TMS trains differed between the two sessions locally in the ipsilesional sensorimotor cortex (SM1) and almost significantly after correction for multiple comparisons in the distal contralesional M1. This possible change in effective connectivity of the ipsilesional M1 with the contralesional M1 could reflect a change in the strength of interhemispheric interactions that are important normally for the coordination of hand movements (Ferbert et al., 1992). Direct connections between the M1s in the two hemispheres appear to exist in the human. In healthy individuals, TMS applied over M1 in one hemisphere reduces motor potentials in the hand evoked by TMS applied 6–11 ms later over the opposite M1 (Ferbert et al., 1992; Di Lazzaro et al., 1999). Studies combining TMS and PET report changes in cerebral activity in the right M1 as the result of repetitive TMS applied over the left M1 (Fox et al., 1997; Paus et al., 1998; Siebner et al., 2000; Chouinard et al., 2003). In normal volunteers, repetitive TMS applied over M1 disrupts finger sequences as subjects play the piano with either hand (Chen et al., 1997). Cerebral activity also increases in the M1 ipsilateral to the hand that performs a complex task compared with a simpler task (Rao et al., 1993; Shibasaki et al., 1993). In recovered stroke patients, similar increases in the contralesional
M1 occur during simple hand movements performed by the recovered arm (Chollet et al., 1991; Weiller et al., 1992, 1993; Honda et al., 1997; Cao et al., 1998). The analogy in the level of involvement of the M1 ipsilateral to the arm performing complex hand movements by healthy volunteers and simple hand movements by recovered stroke patients suggests that these patients recruit resources in the intact hemisphere to fulfill motor tasks.

**Changes in the effective connectivity of M1**

Our results revealed changes in the CBF response in the cingulate motor area (Paus, 2001; Paus et al., 1993; Picard and Strick, 1996) when applying TMS over the ipsilesional SM1. This cingulate motor area represents most likely the human caudal cingulate zone, which is located posterior to the anterior commissure (Paus, 2001; Paus et al., 1993; Picard and Strick, 1996). The cortical motor system is hierarchically organized with M1 executing movements via its direct influence on the spinal motor-neurons. The non-primary motor areas with a much weaker influence on the spinal motor-neurons (Maier et al., 2002; Lemon et al., 2002) are responsible normally for the planning, selection, and maintenance of movements (Ashe and Ugurbil, 1994). The cingulate motor areas are involved normally during motor tasks that require a greater level of voluntary control (Paus, 2001; Paus et al., 1993; Picard and Strick, 1996). We speculate therefore that this change in the cingulate motor area reflects a strengthening of connections to recruit resources to fulfill motor tasks that were previously automatic and/or effortless before stroke.

Changes in effective connectivity of M1 with the basal ganglia and thalamus could be related to the role of the cortico-basal ganglia–thalamo–cortical loops in processing information related to the control of movement and learning motor sequences (Parent and Hazrati, 1995; Doyon et al., 2003). Patients in this study demonstrated greater improvements in the functional use of the affected arm in everyday life and in coordinating sequences of movements rather than in making simple repetitive movements. These improvements correlated further with post–pre CBF differences in the putamen, globus pallidus, and the VL thalamus. In addition, studies performed by Grafton et al. (1994) and Doyon et al. (2002) have demonstrated changes in cerebral activity in the putamen of healthy volunteers that coincide with long-lasting retention of acquired motor skills. It is conceivable therefore that changes in the effective connectivity of M1 with the basal ganglia and thalamus could reflect a reorganization of the cortico-basal ganglia–thalamo–cortical loop necessary to perform and retain newly acquired skills.

**Concluding remarks**

Functional imaging studies have demonstrated altered cerebral activity in motor regions of the brain in recovered stroke patients who execute movements with their affected arm (e.g., Chollet et al., 1991; Weiller et al., 1992, 1993; Cramer et al., 1997; Dettmers et al., 1997; Honda et al., 1997; Cao et al., 1998; Seitz et al., 1998; Nelles et al., 2001; Ward et al., 2003a,b). A small number of studies also describe correlates between motor improvements after rehabilitative therapy and altered fMRI activity (Levy et al., 2001; Johansen-Berg et al., 2002). Most of these studies, however, do not discern how different brain regions influence each other. The results presented here provide complimentary insight into rehabilitation-mediated recovery by demonstrating changes in the effective connectivity of the motor system after CI therapy.

**Acknowledgments**

We thank Dr. Liam Durcan for conducting the neurological examinations, Dr. Nancy Mayo and Mrs. Lois Finch for helping us with patient recruitment, Dr. Edward Taub (University of Alabama at Birmingham) for allowing the first author to learn about constraint-induced movement therapy, and Mrs. Oriane Landry for statistical advice. This work was supported by the Canadian Institutes of Health Research (MT-14505) and by the Canadian Foundation for Innovation.

**References**


