Bilateral Priming Accelerates Recovery of Upper Limb Function After Stroke
A Randomized Controlled Trial

Cathy M. Stinear, PhD; Matthew A. Petoe, PhD; Samir Anwar, FAFRM (RACP); Peter Alan Barber, FRACP; Winston D. Byblow, PhD

Background and Purpose—The ability to live independently after stroke depends on the recovery of upper limb function. We hypothesized that bilateral priming with active–passive movements before upper limb physiotherapy would promote rebalancing of corticomotor excitability and would accelerate upper limb recovery at the subacute stage.

Methods—A single-center randomized controlled trial of bilateral priming was conducted with 57 patients randomized at the subacute stage after first-ever ischemic stroke. The PRIMED group made device-assisted mirror symmetrical bimanual movements before upper limb physiotherapy, every weekday for 4 weeks. The CONTROL group was given intermittent cutaneous electric stimulation of the paretic forearm before physiotherapy. Assessments were made at baseline, 6, 12, and 26 weeks. The primary end point was the proportion of patients who reached their plateau for upper limb function at 12 weeks, measured with the Action Research Arm Test.

Results—Odds ratios indicated that PRIMED participants were 3× more likely than controls to reach their recovery plateau by 12 weeks. Intention-to-treat and per-protocol analyses showed a greater proportion of PRIMED participants achieved their plateau by 12 weeks (intention to treat, \( \chi^2=4.25; P=0.039 \) and per protocol, \( \chi^2=3.99; P=0.046 \)). ANOVA of per-protocol data showed PRIMED participants had greater rebalancing of corticomotor excitability than controls at 12 and 26 weeks and interhemispheric inhibition at 26 weeks (all \( P<0.05 \)).

Conclusions—Bilateral priming accelerated recovery of upper limb function in the initial weeks after stroke.

Clinical Trial Registration—URL: http://www.anzctr.org.au. Unique identifier: ANZCTR1260900046822.

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Key Words: motor evoked potentials ■ neuronal plasticity ■ neurophysiology ■ physical therapy techniques ■ rehabilitation ■ transcranial magnetic stimulation, single pulse ■ upper extremity

The ability to live independently after stroke depends on the recovery of motor function, particularly of the upper limb. The potential for recovery is related to the extent of cerebral damage that creates a ceiling effect, with a plateau usually reached within 6 months after stroke. There are no treatments available that can repair the stroke lesion and raise the recovery ceiling. An alternative strategy could be the development of adjuvant techniques that accelerate recovery and help patients more efficiently reach a plateau of best possible function.

Techniques that prime the brain for a more plastic response to therapy may accelerate motor recovery after stroke. Increasing excitability and reducing inhibition are important precursors for neural plasticity, which may allow surviving neural elements to more easily reorganize in response to therapy. Active–passive bilateral priming (APBP) is a pattern of coordinated movement that disinhibits the M1 contralateral to the assisted (paretic) limb and facilitates its excitability for ≥30 minutes after a 15-minute session. In patients with ≥6 months after stroke, daily APBP followed by motor practice led to increased ipsilateral corticomotor excitability and a greater improvement in upper limb function compared with motor practice alone. The aim of this study was to determine the immediate and longer term effects of bilateral priming with patients with stroke at the subacute stage. We hypothesized that APBP before upper limb therapy would accelerate the recovery of hand and arm function, with a greater proportion of PRIMED participants reaching maximum recovery by 12 weeks.

Methods

Participants
Consecutive patients aged ≥18 years admitted with first-ever monolateral ischemic stroke were screened between November 2009
and March 2012 (Figure 1). Patients were excluded if they did not require upper limb rehabilitation or were unsuitable because of spasticity, homonymous hemianopia, blindness, visuospatial neglect, or complete somatosensory loss. Patients were also excluded if they were unsuitable for research participation because of contraindications to transcranial magnetic stimulation or MRI and cognitive or communication impairment or pre-existing conditions precluding informed consent or compliance with study assessments. The study was approved by the regional ethics committee, and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Assessments
Participants completed baseline assessments with the Action Research Arm Test (ARAT),10 the Fugl–Meyer scale, and the National Institutes of Health Stroke Scale to evaluate baseline upper limb function, impairment, and stroke severity, respectively. Clinical assessments were repeated after the 4-week intervention and at 12 and 26 weeks after stroke. The modified Rankin Scale and Stroke Impact Scale were used to evaluate disability and quality of life at 26 weeks. Participants were assessed by therapists who were unaware of their group allocation and did not treat them.

Figure 1. Trial profile. APBP indicates active–passive bilateral priming; TIA, transient ischemic attack; and TMS, transcranial magnetic stimulation.
Randomization
Participants were randomized and began the intervention within 26 days of stroke. Intervention allocation was concealed and randomized using customized software (www.rando.la) that minimized between-group differences in age, baseline ARAT score, PREP stratification, and brain-derived neurotrophic factor genotype derived from a single baseline blood sample because this may influence plasticity and learning. To stratify patients using the PREP algorithm, we first graded shoulder abduction and finger extension strength of the paretic upper limb 72 hours after stroke. Next, transcranial magnetic stimulation was used to determine whether motor evoked potentials could be recorded from the extensor carpi radialis (ECR) muscle of the paretic upper limb. Finally, T1-weighted and diffusion-weighted images were acquired with a Siemens 1.5 T Avanto scanner (Methods in the online-only Data Supplement). The mean fractional anisotropy (FA) was calculated within the posterior limb of each internal capsule. The structural integrity of the posterior limbs of the internal capsules was quantified by calculating an asymmetry index from the mean FA values: \( FA_{ASYM} = \frac{FA_{contra} - FA_{ipsi}}{FA_{contra} + FA_{ipsi}} \).

Neurophysiological Measures
Transcranial magnetic stimulation was used to evaluate corticomotor excitability in each hemisphere and interhemispheric inhibition. Motor evoked potentials were recorded from the ECR of the paretic upper limb using standard surface electromyography techniques. Rest motor threshold was determined for each ECR and stimulus–response (S–R) curves constructed by recording blocks of 12 motor evoked potentials at intensities −5%, +5%, +15%, +25%, and +35% of maximum stimulator output relative to rest motor threshold, with the order of stimuli intensities randomized.

Interhemispheric inhibition was evaluated by delivering single magnetic stimuli at 80% maximum stimulator output to each M1 while participants maintained full voluntary extension of the ipsilateral wrist against gravity. In healthy adults, this protocol produces a period of silence in the electromyography of ipsilateral ECR that begins \( \approx 30 \) ms after stimulation and lasts for 30 to 50 ms. The ipsilateral silent period reflects the excitability of interhemispheric pathways responsible for inhibition passed between the motor cortices (Methods in the online-only Data Supplement).

Interventions
Participants allocated to the bilateral priming group (PRIMED) used a portable device to couple the 2 hands mechanically and produce rhythmic, continuous bimanual mirror symmetrical movements for 15 minutes. Participants actively flexed and extended the nonparetic wrist, with the device driving the paretic wrist in a mirror symmetric pattern (Figure I in the online-only Data Supplement). The device confers an inertial advantage such that little force is required from the active wrist. Participants were instructed to move at a comfortable rate and were given a target of 500 to 1500 movement cycles, depending on their individual ability. The number of movement cycles completed each session was recorded from a digital counter on the device.

The CONTROL intervention (CONTROL) was intermittent cutaneous electric stimulation of the volar aspect of the paretic forearm, using a standard TENS unit delivered for 15 seconds (including 2-s ramp-up, 2-s ramp-down), once per minute, for 15 minutes. The intensity was adjusted to produce a mild cutaneous sensation for each participant and served only to control for participants’ expectations. The priming and control interventions were delivered immediately before upper limb therapy every weekday for 4 weeks. Participants continued self-directed priming and therapy at home if they were discharged from inpatient rehabilitation during the 4-week intervention period. Although participants could not be blinded to the priming, they had no reason to expect one technique was more effective.

Table 1. Baseline Characteristics for Randomized Patients

<table>
<thead>
<tr>
<th>Factor</th>
<th>PRIMED</th>
<th>CONTROL</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>29</td>
<td>28</td>
<td>...</td>
</tr>
<tr>
<td>Age (y)</td>
<td>68 (33–97)</td>
<td>71 (31–90)</td>
<td>0.46</td>
</tr>
<tr>
<td>Men</td>
<td>11 (38%)</td>
<td>15 (54%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Clinical characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke severity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIHSS score</td>
<td>4 (1–12)</td>
<td>4 (0–17)</td>
<td>0.85</td>
</tr>
<tr>
<td>Upper limb FM score</td>
<td>44 (2–65)</td>
<td>43 (3–64)</td>
<td>0.82</td>
</tr>
<tr>
<td>ARAT score</td>
<td>26 (0–56)</td>
<td>27 (0–57)</td>
<td>0.82</td>
</tr>
<tr>
<td>Stroke lesion characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant hemisphere</td>
<td>13 (45%)</td>
<td>10 (30%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Motor cortex</td>
<td>4 (14%)</td>
<td>2 (7%)</td>
<td>0.67</td>
</tr>
<tr>
<td>Subcortical white matter</td>
<td>18 (62%)</td>
<td>24 (86%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>10 (34%)</td>
<td>15 (54%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Brain stem</td>
<td>7 (24%)</td>
<td>2 (7%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Intravenous thrombolysis</td>
<td>2 (7%)</td>
<td>0</td>
<td>0.49</td>
</tr>
<tr>
<td>Factors affecting upper limb recovery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticospinal tract integrity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEPs present</td>
<td>22 (76%)</td>
<td>21 (75%)</td>
<td>0.94</td>
</tr>
<tr>
<td>FA asymmetry</td>
<td>0.074 (-0.062–0.418)</td>
<td>0.049 (-0.117–0.393)</td>
<td>0.46</td>
</tr>
<tr>
<td>Predicted upper limb recovery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>9 (31%)</td>
<td>9 (32%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Notable</td>
<td>13 (45%)</td>
<td>12 (43%)</td>
<td></td>
</tr>
<tr>
<td>Limited or none</td>
<td>7 (24%)</td>
<td>7 (25%)</td>
<td></td>
</tr>
<tr>
<td>BDNF genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>val/val</td>
<td>17 (59%)</td>
<td>16 (57%)</td>
<td>0.99</td>
</tr>
<tr>
<td>val/met</td>
<td>10 (34%)</td>
<td>10 (30%)</td>
<td></td>
</tr>
<tr>
<td>met/met</td>
<td>2 (7%)</td>
<td>2 (7%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are median (range) or number (%). Predicted upper limb recovery is based on the PREP algorithm. ARAT indicates Action Research Arm Test; BDNF, brain-derived neurotrophic factor; FM, Fugl–Meyer; MEP, motor evoked potential; and NIHSS, National Institutes of Health Stroke Scale.
Upper Limb Rehabilitation

A standardized dose of upper limb rehabilitation began within 4 weeks after stroke, consisting of 30 minutes of physiotherapy and occupational therapy delivered every weekday for 4 weeks. Therapy was delivered immediately after the completion of either the priming or control intervention by therapists who were unaware of group allocation. The amount of time spent priming and then in therapy was recorded for each session by therapists or by the participant using a therapy diary if they completed self-directed priming and therapy at home. Compliance was monitored with telephone calls and home visits, and diaries were returned at the end of the intervention for analysis. Participants also received standard care throughout the study.

Statistical Analysis

Baseline Measures

Independent 2-sided t tests were used for linear continuous variables. Two-sided Pearson χ² tests were used for nominal and ordinal variables, except when cell counts were <5, in which case 2-sided Fisher exact tests were used.

Primary End Point

Participants were binarized at 12 weeks according to whether they had reached their recovery plateau, defined as achieving ≥75% of their maximum recovery on the ARAT score or being within 1 point of their maximum ARAT score. This criterion was appropriate because the average recovery across all participants was 74% at this time. We tested whether a greater proportion of PRIMED than CONTROL participants achieved their plateau using 2-sided Pearson χ² tests. Per-protocol and intention-to-treat analyses were performed, and odds ratios were calculated. ARAT scores from all randomized patients, compared with CONTROL participants (intention-to-treat (23/29 PRIMED; 15/28 CONTROL; χ²=3.99; P=0.046). Primed participants were 3× more likely to achieve their plateau within 12 weeks compared with CONTROL participants (intention-to-treat odds ratio, 3.32; 95% confidence interval, 1.0–10.7 and per-protocol odds ratio, 3.54; 95% confidence interval, 1.0–12.6).

Secondary Clinical Measures

At 26 weeks, there were no between-group differences in median modified Rankin Scale score (2; PRIMED range, 0–4; CONTROL range, 0–5; Mann–Whitney U test; P=0.4) or mean Stroke Impact Scale score (63.5; SE, 2.2; CONTROL, 65.4; SE, 2.4; t test P>0.2).

Neurophysiological Measures

There were no differences between the PRIMED and CONTROL groups at baseline (t tests; all P>0.15; Table I in the online-only Data Supplement).

Corticomotor Excitability

Bilateral priming promoted rebalancing of corticomotor excitability (Figure 2B and 2C). The S–R slope was used as a measure of corticomotor excitability in each hemisphere. There was a main effect of hemisphere and an interaction between hemisphere and time on S–R slope (Table I in the online-only Data Supplement). There was an interaction among group, hemisphere, and time, which was decomposed with RM ANOVA for each group. There were main effects of hemisphere for both groups, but only the PRIMED group had an interaction between hemisphere and time. This expected effect arose because ipsilesional slope increased, and contralateral slope decreased, over time in the PRIMED group but not in the CONTROL group.

Interhemispheric Inhibition

Bilateral priming increased the excitability of transcortical projections from the ipsilesional to contralesional M1. The excitability of these projections was indexed by the persistence and depth of ipsilateral silent periods produced in the ongoing electromyography recorded from the nonparetic ECR by stimulation of ipsilesional M1. As expected, both the

<table>
<thead>
<tr>
<th>Table 2. Protocol Compliance</th>
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<tbody>
<tr>
<td>PRIMED</td>
</tr>
<tr>
<td>Priming</td>
</tr>
<tr>
<td>Enrolment, d.p.s.</td>
</tr>
<tr>
<td>Initiation, d.p.s.</td>
</tr>
<tr>
<td>Priming, total d</td>
</tr>
<tr>
<td>Duration, min/d</td>
</tr>
<tr>
<td>Duration, total min</td>
</tr>
<tr>
<td>Intensity, per d</td>
</tr>
<tr>
<td>Therapy</td>
</tr>
<tr>
<td>Duration, min/d</td>
</tr>
<tr>
<td>Duration, total min</td>
</tr>
<tr>
<td>Assessments</td>
</tr>
<tr>
<td>Baseline, d.p.s.</td>
</tr>
<tr>
<td>Postintervention, d.p.s.</td>
</tr>
<tr>
<td>Primary end point, d.p.s.</td>
</tr>
<tr>
<td>Follow-up, d.p.s.</td>
</tr>
</tbody>
</table>

All data are median (range) and were analyzed with independent samples Mann–Whitney U tests. Intensity refers to the number of cycles of active–passive bimanual movement completed in a single therapy session for the PRIMED group, and the current of the transcutaneous stimulation applied in the CONTROL group. d.p.s. indicates days post stroke.
persistance and depth of ipsilateral silent periods produced by stimulation of the ipsilesional M1 increased over time for the PRIMED group, but perhaps surprisingly decreased over time for the CONTROL group (Table I and Figure II in the online-only Data Supplement).

Discussion

This is the first study to show that bilateral priming before upper limb therapy accelerates the recovery of upper limb function after stroke and increases the odds of reaching the recovery plateau by 12 weeks for equivalent therapy dose. Bilateral priming was suitable for 80% of patients with upper limb weakness and is feasible in the clinical setting. Recovery of motor function usually plateaus during the first 6 months after stroke, and prestroke levels of function are seldom restored.4 Conceptually, aiming to accelerate the rate of recovery during rehabilitation may be a more realistic goal than attempting to overcome the recovery ceiling.

The neurophysiological effects of APBP are the most likely mechanism underlying the observed acceleration of recovery. Excitability increased for both descending and transcallosal projections from the ipsilesional M1 in the PRIMED group but not in the CONTROL group. These effects were evident 12 and 26 weeks after stroke, indicating long-term benefits for the motor system, which may overcome a progressive neurophysiological decline (CONTROL ipsilateral silent period; Figure II in the online-only Data Supplement). Bilateral priming facilitates corticomotor excitability in the hemisphere contralateral to the assisted (paretic) upper limb for ≥30 minutes.8 This period of disinhibition7 may create a therapeutic window where plastic reorganization within the ipsilesional M1 is more likely to occur. It is unlikely that APBP constitutes an additional 15 minutes of therapy because it is not task-specific and can be completed without any active movement of the paretic upper limb. Furthermore, a 15-minute increase in daily upper limb therapy has no beneficial effects.16 Bilateral priming is a neuromodulatory adjuvant rather than a therapy and is, therefore, distinct from bilateral isokinematic training or bilateral arm training with auditory cueing,17,18 which have been found to have no additional benefits by a recent Cochrane review.19

This study has a number of potential limitations. First, the sample of 57 patients is relatively small. Sample size was limited by the neurophysiological and neuroimaging techniques used to stratify patients for research purposes, even though APBP was suitable for most patients. Second, although the duration of each therapy session was recorded, it is difficult to measure self-directed practice completed by patients during rehabilitation after stroke. This is a challenge for all rehabilitation trials and not unique to this study. Although participants are likely to have completed a wide range of total therapy doses, the randomization process makes systematic between-group differences unlikely.

This study also has a number of strengths. All participants began the intervention within 26 days of stroke, much earlier than the majority of motor rehabilitation randomized controlled trials.20 The groups’ clinical baseline characteristics were well balanced. Blinding was carefully maintained by having 3 groups of therapists who PRIMED, treated, and assessed participants. The successful PRIMED treatment of a heterogeneous sample of patients in a busy clinical setting

Figure 2. A, Primary end point. Intention-to-treat analysis of the proportion of patients who reached their recovery plateau at 6, 12, and 26 weeks after intervention. A significantly greater proportion of the PRIMED patients achieved the plateau at 12 weeks, the primary end point. *P=0.039. Error bars=SD. B and C, Corticomo-

indicates that bilateral priming is feasible in the real world, and that these findings are generalizable.

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Disclosures
Dr Stinear has been funded for travel by Pierre Fabre Pharmaceuticals and receives funding from Health Research Council of New Zealand (11/270). Dr Byblow is a named inventor on a patent for a training device assigned to Uniservices Ltd and receives funding from Health Research Council of New Zealand (11/270). Dr Byblow is a named inventor on a patent for a training device assigned to Uniservices Ltd and receives funding from Health Research Council of New Zealand (11/270). The other authors report no conflicts.

References
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Online Supplement

**Bilateral priming accelerates recovery of upper limb function after stroke: A randomized controlled trial**

In healthy adults, repetitive active-passive bimanual movement facilitates corticomotor excitability, reduces interhemispheric inhibition, and improves motor learning. Active-passive bilateral priming (APBP, or bilateral priming) can be performed easily and repeatedly using a table-top device that mechanically couples the two hands (Figure 1). The user rhythmically flexes and extends one wrist, while the device assists movement of the opposite wrist in a mirror-symmetric pattern. This particular pattern of coordinated movement disinhibits the M1 contralateral to the assisted (paretic) limb, facilitating its excitability for at least 30 minutes after 15 minutes of active-passive movement.

**Supplemental Methods**

*Procedures*

Neurophysiological measures

Recording electrodes (Ambu, Denmark) were placed over the ECR in a belly-tendon montage and the reference electrode (Red Dot, 3M) was placed on the lateral epicondyle of the humerus. Signals were sampled at 2 kHz, amplified (1000 gain), filtered (20 Hz – 1000 Hz), and stored for offline analysis using Signal software (CED, Cambridge). Trial length was 300 ms, with 100 ms of background EMG recorded prior to the stimulus. Magnetic stimuli were delivered using a 70 mm figure-of-eight coil connected to a MagStim 200 unit. The coil was oriented to induce posterior-to-anterior current flow in each M1. RMT was defined as the minimum stimulus intensity that produced a MEP with amplitude ≥ 50 µV on at least 4 out of 8 consecutive trials.
RMT was set to 100% maximum stimulator output (MSO) for participants who did not meet this criterion.

For stimulus-response curves, background muscle activity was monitored and trials discarded online when the root mean square EMG calculated in a 100 ms window prior to the stimulus exceeded 10 µV. MEPs were subsequently processed by first discarding the two smallest and two largest MEPs recorded at each stimulus intensity, to remove any outliers. MEP area was calculated in a 20 ms window for each trial and averaged for each stimulus intensity. A stimulus-response (S-R) curve was then constructed for each muscle in each session, and the slope of the curve was estimated by determining the best linear fit for the linear portion of the curve. The slope of the S-R curve is considered the most robust measure of excitability for the corticomotor pathway.\(^3,4\)

For ipsilateral silent periods, 20 stimuli were delivered to each M1 while participants maintained full voluntary extension of the ipsilateral wrist against gravity, with rests provided between stimuli as required. The persistence of the ipsilateral silent period (iSP) was the percentage of trials that produced an iSP, where mean post-trigger EMG fell below mean pre-trigger EMG for at least 10 ms. Traces were rectified then averaged, and mean pre-trigger EMG was calculated in a 30 ms window beginning 60 ms prior to the magnetic stimulus. Post-trigger EMG was calculated in a window 30 – 60 ms after the magnetic stimulus. The iSP onset was defined as the time at which the rectified post-stimulus EMG fell below mean pre-trigger EMG, for at least 10 ms. The depth of the iSP was calculated with the formula: depth (%) = 100 – ((post\_mean / pre\_mean) x 100), where pre\_mean and post\_mean are the mean pre-trigger and post-trigger EMG described above, averaged over all 20 trials.

Imaging measures
Axial T1-weighted images were used to identify lesion location and had 1.0 x 1.0 x 1.0 mm voxels, a 256 mm field of view, TR = 11 ms, and TE = 4.94 ms. Diffusion-weighted images had 1.8 x 1.8 x 3.0 mm voxels, a 230 mm field of view, b = 2000 s.mm$^2$, TR = 6700 ms, TE = 101 ms, 30 gradient directions and two averages. All image processing was carried out with the Oxford FMRIB Software Library.$^5$

Randomisation and masking

The PREP algorithm$^6,7$ can be used to predict potential for recovery of upper limb function, and begins by grading shoulder abduction and finger extension strength, using the Medical Research Council grades, 72 hours after stroke. If the sum of these two scores (the ‘SAFE’ score) was 8 or more, the patient could be expected to make a complete recovery. Participants with a SAFE score less than 8 in whom TMS could elicit MEPs in the paretic ECR could be expected to make a notable recovery. Participants in whom TMS could not elicit an MEP in the paretic ECR could be expected to make a limited recovery if their PLIC FA asymmetry was less than 0.15, or have no recovery potential if their PLIC FA asymmetry was more than 0.15. Custom software minimized baseline differences on each stratification type when randomizing participants between the PRIMED and CONTROL groups. BDNF genotyping was also undertaken for the purposes of minimizing baseline differences between the PRIMED and CONTROL groups.

There were three groups of therapists involved in the study. The therapists who delivered the priming did not treat or assess the participants. The therapists who treated the participants immediately after each priming session were blinded to their group allocation and the results of the participants’ clinical, radiological, genotyping and neurophysiological assessments. The
therapists who performed the clinical assessments were blinded to participants’ group allocation and were based at a separate site, so they remained blinded to each participant’s progress in rehabilitation. These therapists were also blinded to the results of participants’ clinical, radiological, genotyping and neurophysiological assessments. The experimenters who performed the neurophysiological and imaging assessments, and data processing, were blinded to group allocation and clinical assessments. The intervention was delivered in a separate room, so participants did not see any other patient receive either the priming or control intervention.

Interventions

For the bilateral priming intervention, participants actively flexed and extended the non-paretic wrist through excursions of up to 140 degrees, with the device driving the paretic wrist in a mirror-symmetric pattern (Figure I). The device confers an inertial advantage such that little force is required from the active wrist to maintain repetitive flexion-extension of both wrists. Participants were instructed to make the movements at a comfortable rate, and were given a target of 500 – 1,500 movement cycles, depending on their individual ability and stage of progress, as prescribed by the therapist responsible for priming. Each device had a digital counter, and the number of movement cycles completed in each session was recorded by the priming therapist. During the first week, participants were instructed to keep the paretic upper limb completely relaxed, to minimize interference between between the strong and weak side and to maximize the number of repetitions made. Participants who recovered some active wrist movement in the paretic limb were encouraged to assist with their paretic limb, from the second week onwards, provided that this did not reduce the number of repetitions they could perform. The number of repetitions per session is reported in Table 2. EMG was not recorded during
bilateral priming so the extent of muscle activation on the passive side, or how this varied across participants and sessions, is not known. The important aspect of bilateral priming is that several hundred mirror symmetric movements are produced in order to induce the expected neurophysiological effects.¹

For the control intervention, a pair of 5 cm x 5 cm self-adhesive electrodes were placed over the forearm flexors, approximately 5 cm apart. A transcutaneous electrical stimulation unit (NeuroTrac Rehab, Verity Medical, UK) was used to deliver a weak direct current for 15 seconds (including 2 s ramp up, 2 s ramp down), once per minute, for 15 minutes. The stimulus intensity was adjusted to produce a mild cutaneous sensation for each participant, and the cathode was arbitrarily positioned either proximal or distal. This stimulation would produce no neurophysiological effects and served only to control for participants’ expectations.

Statistical Analysis

Neurophysiological measures: Root mean squared pre-trigger EMG during collection of the S-R curves and iSPs, and the percentage of trials rejected due to excess pre-trigger EMG during S-R curve trials, were analysed using RM ANOVA with between-subject factors Group (PRIMED, CONTROL), Hemisphere (IPSILESIONAL, CONTRALESIONAL) and Time (2, 6, 12, 26 weeks) as the within-subject factors. An alpha of 0.05 was adopted for statistical significance. Huynh-Feldt corrections were used for non-spherical data. Two-tailed \( t \)-tests were used for post-hoc comparisons with a modified Bonferroni correction for multiple comparisons. ⁹ Data are reported in the text as mean with standard deviation (SD) or standard error of the mean (SEM). The effects of bilateral priming are the focus of the present report. Any additional effects of PREP stratification and BDNF genotype on recovery of upper limb function will be reported separately.
Supplemental Results

Neurophysiological Measures

Rest Motor Threshold: There were main effects of Hemisphere and Time on RMT, and an interaction between them, as expected (Table I). This arose because ipsilesional RMT at 6, 12 and 26 weeks was lower than at 2 weeks (two-tailed paired t-tests, all \( P < 0.001 \)). Ipsilesional RMT at 12 and 26 weeks was also lower than at 6 weeks (6 weeks 56\% \text{ SEM} 3\% \text{ MSO}; 12 weeks 53\% \text{ SEM} 3\% \text{ MSO}, \( t_{46} = 2.15, P = 0.037 \); 26 weeks 52\% \text{ SEM} 3\% \text{ MSO}, \( t_{46} = 2.80 \ P = 0.007 \)). There was no difference in ipsilesional RMT between 12 and 26 weeks (\( t_{46} = 1.87, p = 0.068 \)). There was no change in contralesional RMT over time (all \( P > 0.35 \)). There were no interactions with Group (all \( P > 0.065 \)).

Corticomotor excitability: Ipsilesional S-R slope increased, and contralesional S-R slope decreased, over time in the PRIMED group but not the CONTROL group (Table I, Figure 2 B,C). For the PRIMED group, ipsilesional slope was steeper at 12 and 26 weeks than at 2 weeks (12 weeks 0.20 s.d. 0.07 mV/10\%MSO, \( t_{24} = 2.34, P = 0.028 \); 26 weeks 0.28 s.d. 0.08 mV/10\%MSO, \( t_{24} = 2.36, P = 0.027 \)). Contralesional slope was flatter at 26 weeks than at 2 weeks (26 weeks 0.82 s.d. 0.09 mV/10\%MSO, \( t_{24} = 2.82, P = 0.010 \)).

EMG level: Both ECR muscles were at rest during data collection. The mean percentage of trials discarded due to excess pre-trigger EMG was 1.7\% \text{ SEM} 0.4\%. Mean pre-trigger rmsEMG for analysed trials was 4.8 \( \mu \text{V SEM} \) 0.2 \( \mu \text{V} \).

Interhemispheric inhibition: Data were analysed for 30 of 57 participants (15 in each Group), as 27 were unable to maintain paretic wrist extension against gravity at baseline, a requirement for recording iSPs.
For the persistence of iSPs, there was an interaction between Group, Hemisphere and Time, which was decomposed with RM ANOVA for each Hemisphere (Table I). There was an interaction between Group and Time for the ipsilesional hemisphere but not the contralesional hemisphere (Figure II A,B). The interaction arose because the persistence of iSPs produced by stimulation of the ipsilesional M1 increased over Time for the PRIMED group and decreased over Time for the CONTROL group, becoming significantly different by 26 weeks (PRIMED 87% SEM 4%, CONTROL 65% SEM 7%, $t_{28} = 2.78, P = 0.011$).

For iSP depth, there was a main effect of Time and an interaction between Hemisphere and Time, which was decomposed with RM ANOVA for each Hemisphere (Table I). There was an interaction between Group and Time for the ipsilesional hemisphere but not the contralesional hemisphere (Figure II C,D). The interaction arose because the depth of iSPs produced by stimulation of the ipsilesional M1 increased over Time for the PRIMED group, and decreased over Time for the CONTROL group, becoming significantly different by 26 weeks (PRIMED 54% SEM 3%, CONTROL 44% SEM 3%, $t_{28} = 2.31, P = 0.028$).

For background rmsEMG in the ECR ipsilateral to stimulation, there was a main effect of Hemisphere ($F_{1,28} = 50.30, P < 0.001$) and an interaction between Hemisphere and Time ($F_{3,84} = 8.38, P < 0.001$), as expected. The interaction arose because mean pre-trigger rmsEMG in the paretic ECR during contralesional M1 stimulation increased over time (baseline 96 mV SEM 9 mV; 6 weeks 116 mV SEM 10 mV; 12 weeks 126 mV SEM 9 mV; 26 weeks 143 mV SEM 10 mV; all pairwise $t$-tests with baseline $P < 0.005$). There was no effect of Time on non-paretic ECR background rmsEMG during ipsilesional M1 stimulation (197 mV SEM 12 mV; all $P > 0.08$). Mean latency of the iSP was longer when cM1 was stimulated than iM1 (cM1 41 ms SEM 1 ms; iM1 39 ms SEM 1 ms; $t_{29} = 3.75, P = 0.001$).
Supplemental References


Table I. Neurophysiological results from Transcranial Magnetic Stimulation. Mean ± standard deviation; MSO=maximum stimulator output; mV=milliVolts; S-R=Stimulus-Response curve; iSP=ipsilateral silent period IL=Ipsilesional M1 stimulated; CL=Contralesional M1 stimulated.
**Figure I. Active-passive bilateral priming.** The patient rhythmically flexes and extends the non-paretic wrist, while the device assists movement of the paretic wrist in a mirror-symmetric pattern via a mechanical coupling. The device confers an inertial advantage such that little force is required from the active wrist to maintain repetitive flexion-extension of both wrists. Participants in the PRIMED group completed several hundred movement cycles (see Table 2), during each 15 minute priming session, prior to 30 minutes of upper limb therapy.
Figure II. Interhemispheric inhibition. The persistence (A,B) and depth (C,D) of ipsilateral silent periods elicited by iM1 stimulation increased in the PRIMED group, while depth decreased in the CONTROL group. The persistence and depth of ipsilateral silent periods elicited by contralesional M1 stimulation were stable. In all panels the grey area indicates the 4 week period of primed therapy. Error bars = standard error; * P<0.05, two-tailed t-test comparison between Groups.