Motor cortex excitability in stiff-person syndrome

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Summary
Muscle stiffness in stiff-person syndrome (SPS) is produced by continuous, involuntary firing of motor units that is thought to be caused by an autoimmune mediated dysfunction of GABA-ergic inhibitory neurones. We have postulated that the loss of GABA-ergic inputs from spinal interneurones alone is insufficient to produce tonic firing of motor neurones and that excessive supraspinal excitation could also play a role. To determine whether SPS is associated with dysfunction in supraspinal GABA-ergic neurones, we assessed the excitability of the motor cortex with transcranial magnetic stimulation (TMS) in seven SPS patients and seven age-matched healthy volunteers. SPS patients had normal central motor conduction times, normal thresholds for motor evoked potentials (MEPs) in leg muscles, and a normal MEP stimulus versus response recruitment curve with increasing TMS intensities in resting hand and leg muscles. Cortical silent periods were shortened in leg muscles. Intracortical inhibition and excitation were assessed while recording from the abductor pollicis brevis, using a paired pulse TMS paradigm with subthreshold conditioning stimuli. Patients had decreased inhibition and markedly increased facilitation at short intervals. Using paired suprathreshold TMS, patients exhibited increased facilitation at 20- and 40-ms intervals. These results point to a hyperexcitability of the motor cortex in SPS, which could be explained by impairment of supraspinal GABA-ergic neurones, leading to an impaired balance between inhibitory and excitatory intracortical circuitry.

Keywords: stiff-person syndrome; transcranial magnetic stimulation; intracortical excitation; intracortical inhibition; silent period

Abbreviations: aMT = active motor threshold; APB = abductor pollicis brevis muscle; CMAP = compound muscle action potential; ICE = intracortical excitation; ICI = intracortical inhibition; ISI = interstimulus interval; MEP = motor evoked potential; SPS = stiff-person syndrome; TA = tibialis anterior muscle; TMS = transcranial magnetic stimulation

Introduction
Stiff-person syndrome (SPS) is a rare, acquired disorder with fluctuating muscle stiffness and superimposed spasms (Moersch and Woltmann, 1956). SPS is thought to be an autoimmune disorder because the majority of patients have circulating or intrathecal antibodies that react against the intracellular enzyme glutamic acid decarboxylase (Solimena et al., 1990; Solimena and De Camilli, 1991). The majority of these antibodies inhibit GABA (γ-aminobutyric acid) synthesis in vitro (Dinkel et al., 1998) and could, if internalized by GABA-ergic neurones, impair the production of transmitter and reduce the effectiveness of inhibitory GABA-ergic synapses. Impairment of GABA-ergic inhibition, particularly in the spinal cord, has been hypothesized to bring about the clinical features of muscle stiffness and spasms.

EMG recordings have shown that the muscle stiffness is caused by continuous firing of motor neurones (Meinck et al., 1984). Normally, motor neurones have no resting discharge. The development of a resting discharge in SPS suggests that motor neurones become depolarized. This state could be caused either by loss of tonic inhibitory inputs or by sustained excitatory inputs or both. Measures of motor neurone pool excitability, such as the H_max/M_max ratio, have been normal in SPS patients (Meinck et al., 1984; Correale et al., 1988; Floeter et al., 1998), providing some evidence that the enhanced activity originates at a pre-motoneuronal location. A recent study assessed the excitability of inhibitory spinal cord circuits in patients with SPS and found surprisingly limited abnormalities in reflexes thought to be mediated by GABA-ergic interneurones (Floeter et al., 1998). These data raise the question whether loss of the spinal GABA-ergic inhibition is sufficient to bring about tonic motor neurone firing and muscle stiffness. The alternative hypothesis, that motor neurones receive an excess of excitatory drive, could

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Physiological studies have provided some evidence for hyperexcitability of the brainstem in SPS (Matsumoto et al., 1994; Meinck et al., 1994, 1995), but few studies have assessed the motor cortex in SPS patients. Central motor conduction times have been measured in a few patients and were normal (Meinck et al., 1994; Schulte-Mattler, 1996). One patient was reported to have an abnormally long latency response that was abolished by diazepam treatment (Schulte-Mattler, 1996). Because corticospinal neurones synapse upon several classes of spinal interneurones as well as motor neurones, they are a candidate source of excessive excitatory drive to motor neurones. The excitability of corticospinal neurones is dynamically regulated by intrinsic excitatory and inhibitory cortical networks (Kujirai et al., 1993), including GABA-ergic interneurones that synapse directly upon the soma or initial segments (Hendry et al., 1983; Keller, 1993; DeFelipe, 1999). Dysfunction of intrinsic cortical GABA-ergic neurones might lead to excessive corticospinal activation. To assess the excitability of the motor cortex, we used transcranial magnetic stimulation (TMS) with several different paradigms. Single pulse paradigms were used to assess thresholds, recruitment and cortical silent periods (Fuhr et al., 1991; Cantello et al., 1992; Leis et al., 1993; Davey et al., 1994; Brasil-Neto et al., 1995). Paired pulse TMS was used to test the excitability of intracortical circuits (Claus et al., 1992; Valls-Sole et al., 1992; Kujirai et al., 1993). Preliminary results have appeared in abstract form.

Methods

Subjects
Seventeen patients with SPS (five men, two women), aged 32–55 years, and seven healthy volunteers (four men, three women), aged 34–61 years, participated in the study. The protocol was approved by the Institutional Review Board and all subjects gave their written informed consent according to the declaration of Helsinki. Patients were studied during screening visits prior to enrolment into an experimental treatment protocol. The diagnosis of SPS was based on presentation with axial muscle stiffness with progressive worsening, history of superimposed muscle spasms, a beneficial clinical response to benzodiazepines on at least one occasion, exclusion of myotonic disorders and neuromyotonia by EMG, and serum antibodies that reacted against GABA-ergic neurones on tissue sections (Toro et al., 1994). Most SPS patients had stiffness of proximal leg muscles, with variable stiffness in arm muscles, and most had other autoimmune disorders such as diabetes mellitus (Table 1). Of note is that one patient had a history of a cortical vein thrombosis affecting the left frontoparietal lobe 17 years prior to this study, with few sequelae. In this patient, only the contralateral right motor cortex was studied. All patients continued to receive their regular medication (Table 1) and most took their usual dose of benzodiazepines or baclofen within 2–3 h prior to the study. In spite of the administration of these drugs, all patients were symptomatic with residual muscle stiffness at the time of testing.

Apparatus
In patients with asymmetrical muscle stiffness, testing was performed on the more symptomatic side, otherwise the left-sided extremities were preferred. Subjects sat in a reclining chair with the arm or leg restrained in a device that allowed measurement of the isometric force produced by thumb abdution or foot dorsiflexion. An oscilloscope provided visual feedback of the force level to the subject and examiner, with a target corresponding to 20% of maximal voluntary contraction. The EMG signal was also played through a loudspeaker. This visual and auditory feedback enabled subjects to produce a constant EMG signal when muscle contraction was required and assisted in maintaining complete relaxation at other times. Trials with incorrect relaxation or voluntary contraction were discarded.

Surface EMG was recorded from the abductor pollicis brevis (ABP) or tibialis anterior (TA) muscles with paired 10-mm stainless steel disk electrodes using a counterpoint EMG (Dantec, Campbell, Calif., USA) with filter bandwidth 20 Hz to 2 kHz. The maximal amplitude of the compound muscle action potential (CMAP), the distal motor latency and minimal F-wave latencies were elicited with electrical stimulation of the median nerve near the wrist and the peroneal nerve at the fibular neck. Sweeps of EMG signal were digitized at 10 kHz and saved on a personal computer for off-line analysis using custom software (LabVIEW4, National Instruments, Austin, Tex., USA). Each trace included 100 ms of baseline EMG preceding each stimulus.

Physiological procedures

TMS
Two Magstim 200 stimulators connected to a BiStim module (Magstim, Whitland, UK) were used with a 90-mm round coil (140 mm outer diameter). The coil was placed tangentially over the vertex oriented with the coil current clockwise (as viewed from above) for preferential activation of the right motor cortex, and counterclockwise for the left motor cortex. The optimal position for TMS was determined by varying the position of the coil until the lowest threshold motor evoked potentials (MEPs) were obtained at rest. Position was marked on the scalp to allow the examiner to maintain coil position by matching with markings on the coil. By reducing stimulus intensity in 1% steps from a slightly suprathreshold intensity, measurements of the thresholds for the MEP at rest and with muscle contraction, and the silent period threshold were obtained. MEP thresholds were defined as the lowest
Table 1 Clinical characteristics of patients with SPS

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Symptom duration (years)</th>
<th>Distribution of stiffness</th>
<th>Associated diseases</th>
<th>Medication (daily dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>M</td>
<td>8</td>
<td>Trunk, legs &gt; arms, L &gt; R</td>
<td>Hypothyroidism</td>
<td>Diazepam 30 mg, clonopin 2 mg, baclofen 30 mg, buspirone 5 mg</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>M</td>
<td>26</td>
<td>Trunk, legs</td>
<td>Cortical vein thrombosis, IDDM with enuropathy</td>
<td>Diazepam 15 mg, baclofen 30 mg, phenytoin 200 mg</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>F</td>
<td>3</td>
<td>Trunk, legs &gt; arms</td>
<td>Pernicious anaemia</td>
<td>Lorazepam 2 mg, baclofen 80 mg, prednison 60 mg QOD, Diazepam 10 mg</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>F</td>
<td>20</td>
<td>Trunk, legs</td>
<td>Graves disease</td>
<td>Diazepam 30 mg</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>M</td>
<td>3</td>
<td>Trunk</td>
<td>IDDM (paralysis of hemidiaphragm)</td>
<td>Baclofen 80 mg, dantrelene 100 mg, gabapentin 2400 mg</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>M</td>
<td>2</td>
<td>Trunk, legs</td>
<td>IDDM, B12 deficiency, neuropathy</td>
<td>Diazepam 25 mg</td>
</tr>
<tr>
<td>7</td>
<td>55</td>
<td>M</td>
<td>10</td>
<td>Trunk, legs &gt; arms</td>
<td></td>
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</tr>
</tbody>
</table>

M = male; F = female; L = left; R = right; IDDM = insulin dependent diabetes mellitus.

stimulus level at which five MEPs of at least 50 µV were evoked by 10 consecutive stimuli at rest. The silent period threshold was defined as the lowest stimulus level at which five silent periods, detected by visual inspection, were evoked by 10 consecutive stimuli. The threshold during contraction, the active motor threshold (aMT), was determined as the lowest threshold at which five of 10 stimuli produced MEPs. MEPs were distinguished from background EMG, in part, by the following silent period, and in some cases using an online averager. Thresholds were expressed as percentage of the maximal stimulator output.

**Central motor conduction times**
The ABP and TA MEP latencies were measured during contraction for five traces, using stimulation of 180% of silent period threshold for the APB and 140% of silent period threshold for the TA muscle. Central motor conduction times were calculated using F-waves to calculate the peripheral conduction time (Rossini et al., 1994).

**MEP recruitment measurements**
Magnetic stimulation at increasing intensities was given to construct a stimulus–response recruitment curve. Recruitment curves were obtained at rest and during steady contraction of 20% maximal voluntary contraction. Increments of 20% threshold were used when recording from the APB and 10% for the TA until the maximal stimulator output was reached, beginning from resting motor threshold when recording from relaxed muscles and silent period threshold during contraction. Five MEPs were obtained at each intensity and condition, areas and amplitudes were measured off-line in individual traces, and the average of the five MEP measures used. MEP amplitudes were expressed as the ratio of the maximal CMAP amplitude to allow comparisons between subjects.

**Silent period duration**
Silent periods were measured from the same traces that were used to construct MEP recruitment curves during contraction. Durations were measured from the rectified averages of the five traces at each intensity. Onset was measured from the stimulus artefact. The end of the silent period was defined as that time when the EMG returned to 50% of the average EMG during the 100-ms baseline period. Cursors were calculated automatically using an off-line LabView program.

**Paired pulse TMS**
Intracortical excitability was tested in short and long interstimulus interval (ISI) paired pulse paradigms, recording from the relaxed APB muscle. The short ISI paradigm (Kujirai et al., 1993) used a subthreshold conditioning stimulus 5% below the aMT (absolute percentage of maximal stimulator output), and the intensity of the second (test) stimulus was adjusted to produce MEP amplitudes between 0.5 and 1 mV. Paired stimuli with ISIs of 1, 3, 4, 6, 8, 10, 15 and 20 ms were delivered in a pseudorandomized order in blocks of 20 trials, with an unpaired test stimulus given alone every fourth to fifth stimulation. Trials with background EMG activity were discarded. Long ISI paired TMS experiments used equal suprathreshold conditioning and test stimuli, initially with the muscle relaxed, and then repeated during contraction (Valls-Sole et al., 1992; Wassermann et al., 1996). Conditioning and test stimuli were initially set at 110% of aMT, with adjustments up to 120% to produce test MEP amplitudes around 0.5 mV. The same stimulation intensities were used during contraction. Paired stimuli with ISIs of 20, 40, 70, 100, 130, 160 and 200 ms were given in a pseudorandomized order in blocks of 20 trials, with the test...
alone every fourth to fifth stimulation. Eight recordings were obtained for each interval and condition in the paired pulse experiments. MEPs were measured off-line from individual traces and the mean of the eight was used. The interval between each trial was at least 6 s and TMS was only delivered after background EMG subsided, as monitored on audio speakers.

To assess the possibility that the conditioning TMS pulses produced MEP facilitation by increasing spinal excitability, the effects of conditioning TMS pulses were tested on H-reflexes and MEPs from the flexor carpi radialis muscle (FCR) in interleaved trials in one SPS patient and four normal subjects. The amplitudes of the test MEP and H-reflex were matched, with a target of 0.5–1.5 mV. In most subjects the MEP and H-reflex had the same latency, but in those in whom the response latencies differed by >1 ms, a delay was used to ensure the test responses had the same timing relative to the conditioning pulse. Subthreshold conditioning TMS was tested at 3, 8, 10 and 15-ms ISIs, and suprathreshold conditioning TMS was tested at 20, 40, 100 and 200-ms intervals in the resting FCR muscle.

In one patient (Patient 7) we were not able to elicit any MEPs from the TA muscle even at maximal stimulator output. Two patients did not tolerate testing of MEP recruitment curves in the legs (Patient 2 and 3). These patients were excluded from analysis of leg data.

**Statistical analysis**

$t$-Tests were used to compare measurements between patients and the control group. Slopes of MEP and silent period recruitment curves were calculated by linear regression and compared between patients and controls with two sample $t$-tests. Statistical significance was assumed with $P < 0.05$. ANOVA (analysis of variance) was used to compare curves from paired pulse TMS, and post hoc comparisons using Tukey’s test were used to determine the significant intervals.

For paired pulse experiments with subthreshold conditioning stimuli, we also compared the mean of the combined short ISIs (1–4 ms) of patients versus controls as a measure of intracortical inhibition (ICI) and the means of intervals from 6–20 ms as a measure of intracortical excitation (ICE), an analysis practised by other investigators (Ziemann et al., 1997). This analysis is intended to minimize individual timing differences in the optimal ISIs that assess these two processes. $t$-Tests were used to compare these measures of ICI and ICE between patients and control subjects with significance defined as $P < 0.05$.

**Results**

Patients and control groups were comparable for age, height, MEP latencies and central motor conduction times (Table 2).

**Electrophysiology results**

**Thresholds for MEPs and silent periods**

Patients had higher thresholds than controls for evoking MEPs at rest and during contraction in the hand (Table 2). A similar trend was seen in the leg, but was not significant. The difference between resting and active MEP thresholds was greater in patients than control subjects for both arm and leg. Patients also had higher thresholds for evoking silent periods, but in both patients and controls, the silent period thresholds were just below the active MEP threshold.

**MEP recruitment**

MEP areas and amplitudes were highly correlated ($r = 0.99$) and only the latter will be reported in regard to recruitment. Amplitudes are expressed as the ratio of MEP/CMAP. The maximal MEP/CMAP ratio was not significantly different between patients and controls, indicating that magnetic stimulation was able to activate a comparable proportion of the motor neurone pool during active contraction (Table 2). The slopes of the recruitment curves were not significantly different between patients and control subjects. At rest the TA MEP/CMAP amplitudes were larger in SPS patients than controls, whereas the APB MEP/CMAP amplitudes were smaller in SPS patients, resulting in a parallel shift of the recruitment curves. Because of the variability among patients and the small sample size, these amplitude differences were not significant. Active contraction eliminated amplitude differences, and patient and control MEP recruitment curves superimposed.

**Silent periods**

The silent period in the TA muscle was shorter in patients than controls at all intensities of stimulation (Fig. 1). Silent period durations in the hand were not different.

**Intracortical facilitation and inhibition**

Results of paired pulse testing were similar when calculated from MEP areas or amplitude, and only the area data are reported here. With subthreshold conditioning stimulation, control subjects had reduced MEPs at ISIs of 1, 3 and 4 ms, and increased MEP areas at intervals of 6, 8, 10 and 15 ms, as reported by others (Kujirai et al., 1993; Ridding et al., 1995). SPS patients had slightly less inhibition at the short ISIs (1–4 ms) and markedly increased MEPs at longer ISIs from 6 to 20 ms, which are thought to evaluate intracortical excitatory processes (Fig. 2A and B). ANOVA analysis of the curves showed that patients differed from controls, and post hoc testing identified the ISI of 8 ms as significantly different. At this ISI, the conditioned MEP/test MEP area ratio was twice as large for patients as controls. The analysis in which ISIs were grouped also showed differences between SPS patients and controls for ICI as well as ICE (Fig. 2C).
controls, and the inhibitory phase was delayed and reduced. Increments relative to the silent period threshold (SPT) were four times greater at 20-ms intervals compared with SPS patients. In relaxed muscles, there is normally a biphasic cycle of excitability with initial facilitation of the MEP at an ISI of 20 ms, followed by inhibition around 40 ms with a slower time course of recovery by 200 ms (Claus et al., 1992; Valls-Sole et al., 1992; Wassermann et al., 1996; Chen et al., 1997; Valzania et al., 1997). MEP areas from SPS patients were four times greater at 20-ms intervals compared with controls, and the inhibitory phase was delayed and reduced.

Recovery occurred slowly from 70 to 160 ms, similar to controls (Fig. 3A). When the same paradigm was repeated during muscle contraction, control subjects showed an advance of the inhibitory phase, with a reduced MEP by the 20-ms interval, followed by somewhat more rapid recovery than during muscle relaxation. SPS patients, in contrast, had facilitation of MEPs at the 20-ms ISI, and less inhibition at 40 ms than controls (Fig. 3B). Recovery at the later intervals was similar to control subjects.

In normal subjects, paired TMS with subthreshold conditioning stimuli produced the same pattern and magnitude of inhibition and facilitation for the FCR MEP as for the APB MEP. The conditioning pulse alone produced no change in the FCR H-reflex amplitude, as previously described (Kujirai et al., 1993). With suprathreshold conditioning stimulation, a similar magnitude of inhibition was seen for MEPs and H-reflexes in the FCR muscle as in the APB and there was no significant change in the H-reflex in normal subjects at 20 and 40-ms intervals. In the patient with SPS, the FCR MEP showed marked facilitation with subthreshold stimulation at ISIs of 8 ms and 10 ms, whereas the FCR H-reflex showed relatively little facilitation (Fig. 4A). Similarly, with suprathreshold conditioning stimulation at the ISI of 20 ms, the FCR MEP was markedly facilitated, whereas the FCR H-reflex was unchanged (Fig. 4B).

Analysis of all data was performed including and excluding the one patient with history of contralateral cerebral infarction. This did not alter the finding. The data from this patient was therefore included in the illustrations.

### Discussion

In this study, hyperexcitability of the motor cortex in patients with SPS was demonstrated using TMS. This hyperexcitability was demonstrated mainly in the paired pulse testing at longer ISIs with suprathreshold conditioning stimuli also demonstrated hyperexcitability in SPS patients. In relaxed muscles, there is normally a biphasic cycle of excitability with initial facilitation of the MEP at an ISI of 20 ms, followed by inhibition around 40 ms with a slow time course of recovery by 200 ms (Claus et al., 1992; Valls-Sole et al., 1992; Wassermann et al., 1996; Chen et al., 1997; Valzania et al., 1997). MEP areas from SPS patients were four times greater at 20-ms intervals compared with controls, and the inhibitory phase was delayed and reduced. Recovery occurred slowly from 70 to 160 ms, similar to controls (Fig. 3A). When the same paradigm was repeated during muscle contraction, control subjects showed an advance of the inhibitory phase, with a reduced MEP by the 20-ms interval, followed by somewhat more rapid recovery than during muscle relaxation. SPS patients, in contrast, had facilitation of MEPs at the 20-ms ISI, and less inhibition at 40 ms than controls (Fig. 3B). Recovery at the later intervals was similar to control subjects.

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### Table 2 TMS findings in SPS patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>APB</th>
<th>TA</th>
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<tbody>
<tr>
<td></td>
<td>SPS</td>
<td>Controls</td>
</tr>
<tr>
<td>Thresholds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP Thr (%)</td>
<td>38.6 ± 5.3*</td>
<td>31.3 ± 4.4</td>
</tr>
<tr>
<td>MEPact Thr (%)</td>
<td>41.1 ± 5.8*</td>
<td>33.4 ± 5.0</td>
</tr>
<tr>
<td>MEPrest Thr (%)</td>
<td>55.9 ± 9.9*</td>
<td>40.3 ± 8.0</td>
</tr>
<tr>
<td>MEPrest - MEPact (%)</td>
<td>14.7 ± 6.7*</td>
<td>6.9 ± 3.6</td>
</tr>
<tr>
<td>Latencies and CMCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEPact lat (ms)</td>
<td>21.1 ± 2.6</td>
<td>20.9 ± 2.0</td>
</tr>
<tr>
<td>MEPrest lat (ms)</td>
<td>22.7 ± 2.9</td>
<td>22.3 ± 1.7</td>
</tr>
<tr>
<td>MEPrest lat - MEPact lat</td>
<td>1.6 ± 1.1</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>F-wave (ms)</td>
<td>28.6 ± 2.9</td>
<td>28.0 ± 3.1</td>
</tr>
<tr>
<td>M-wave (ms)</td>
<td>3.6 ± 0.6</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>CMCT (ms)</td>
<td>5.5 ± 1.5</td>
<td>5.6 ± 0.7</td>
</tr>
<tr>
<td>MEP/CMAP amplitudes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMAP (mV)</td>
<td>8.7 ± 4.4</td>
<td>10.4 ± 3.4</td>
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<tr>
<td>MEPmax/CMAP</td>
<td>0.50 ± 0.29</td>
<td>0.42 ± 0.10</td>
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<tr>
<td>Age (years)</td>
<td>47.0 ± 9.1</td>
<td>45.6 ± 7.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175 ± 11.0</td>
<td>176 ± 9.0</td>
</tr>
</tbody>
</table>

Means ± standard deviation; asterisks denote P < 0.05 by t-test; SP = silent period; act = active; CMCT = central motor conduction time.

**Fig. 1** Cortical silent periods in TA muscle of SPS patients (grey bars) and normal subjects (black bars). Means ± standard deviation. Increasing intensity of magnetic stimulation is given in increments relative to the silent period threshold (SPT).
Fig. 2 Paired pulse TMS at short ISIs. Subthreshold conditioning stimulation. (A) Ratio of the conditioned/test areas of the APB MEP at each interval. Mean ± SEM for six SPS patients (open squares) and seven control subjects (black circles). Dashed line indicates the ratio of 1, no change; values above represent facilitation, values below inhibition. The asterisk indicates the interval identified as significant by post hoc Tukey’s test. (B) Values for individual subjects were calculated from the average of eight paired stimuli. Example of conditioned (solid lines) and test (dashed lines) average MEP waveforms for paired stimuli at 3-ms ISI (top pair of traces) and 8-ms ISI (bottom pair of traces) in Patient 6. (C) Measures of intracortical excitability, pooling intervals from 1–4 ms (ICI) and from 6–20 ms (ICE). SPS patients (grey bars) have larger ratios of conditioned/test MEP areas compared with controls (black bars). \( P < 0.05 \). Means ± SD.

Fig. 3 Paired pulse TMS at long ISIs. Suprathreshold conditioning stimulation. Mean ratio of conditioned/test APB MEP areas during muscle relaxation (A) and contraction (B) at ISIs from 20 to 200 ms for six SPS patients (open squares) and seven controls (filled circles). Dashed line indicates a ratio of 1, no change; values above represent facilitation, values below inhibition. Mean ± SEM. A log scale for the ordinate was used in B to accommodate for the full range of data points. Asterisks indicate intervals with significant differences (ANOVA \( P < 0.05 \), Tukey’s test for post hoc comparisons).

TMS paradigms which are thought to assess intrinsic cortical circuitry, rather than in those measures, such as MEP recruitment, that assess the ability of the magnetic stimulator to activate the motor cortex. Excessive facilitation of the APB MEP was seen in paired pulse paradigms that assessed short latency intracortical circuits, as well as in paradigms that assessed longer latency effects. These stimuli did not produce facilitation of H-reflexes, arguing against spinal hyperexcitability as the mechanism producing MEP changes in these paradigms. We interpret this excessive facilitation as resulting from underactivity of cortical inhibitory circuits. There are a number of neuropharmacological studies demonstrating that GABA agonists reduce the normal MEP facilitation in paired pulse TMS at the same intervals in which SPS...
Fig. 4 Effects of TMS conditioning stimuli on FCR MEPs and H-reflexes in an SPS patient. (A) Subthreshold conditioning TMS paradigm. Each panel shows eight superimposed traces, with 100 ms of baseline EMG preceding the stimulus artefact. Upper pair of superimposed traces show the FCR MEP alone (left) and the same response preceded by conditioning TMS 8 ms earlier (right). At this interval, the MEP amplitude is facilitated. Lower pair of superimposed traces show the FCR H-reflex alone (left) and when preceded by conditioning TMS 8 ms earlier (right). The H-reflex amplitude is not significantly different. (B) Suprathreshold conditioning TMS paradigm. Each panel shows eight superimposed traces, with 100 ms of baseline EMG preceding the stimulus artefact. Upper pair of superimposed traces show the FCR MEP alone (left) and the same response preceded by conditioning TMS 20 ms earlier (right). At this interval, the MEP amplitude is markedly facilitated. Lower pair of superimposed traces show the FCR H-reflex alone (left) and when preceded by conditioning TMS 23 ms earlier (right). The H-reflex amplitude is not facilitated.

patients showed excessive facilitation (Inghilleri et al., 1996; Ziemann et al., 1996a). The effects of these and other GABA-enhancing drugs (Ziemann et al., 1996b) on intracortical inhibition and facilitation are opposite to our findings in SPS patients. Thus, excessive facilitation of the MEPs in SPS patients would be compatible with the loss of a normal contribution from inhibitory GABA-ergic circuits.

Paired TMS studies used APB hand muscles because many of our patients had difficulty tolerating TMS at the intensities needed for assessing the more symptomatic leg muscles. However, we did assess also cortical silent periods in leg and hand muscles, and the later portion of this inhibitory phenomenon is thought to be cortically generated (Inghilleri et al., 1993). SPS patients had shorter cortical silent periods in the leg muscles, supporting a more widespread dysfunction of cortical inhibition. There are a number of lines of evidence that paired pulse TMS at the shortest intervals produces inhibition of the MEP by direct inhibition of the corticospinal
neurone. These include epidural recordings that have shown that the subthreshold conditioning stimulus doesn’t itself produce firing of corticospinal neurones, but prevents the volley normally produced by the suprathreshold stimulus (Nakamura et al., 1997; Di Lazzaro et al., 1998). The paired pulse paradigm is a fine-grained method for assessment of intracortical inhibitory circuits in comparison with the measurements of the cortical silent period. A greater sensitivity of paired pulse studies may explain why the clinically unaffected APB muscles showed abnormalities in paired pulse TMS studies, but did not exhibit shortening of the cortical silent periods, which was seen in the more severely affected leg muscles.

It is a limitation of our study that we were unable to withdraw patients from benzodiazepines for testing. In SPS patients, medication withdrawal has the reported risk of symptomatic exacerbation including death (Lorish et al., 1989), and unacceptable symptom exacerbation occurred in our previous experiences with attempts to decrease benzodiazepines in SPS patients. Reducing the dosage also brings about confounding effects of withdrawal. One finding that would be consistent with a medication effect was that SPS patients had higher thresholds for eliciting MEPs, as has been seen with benzodiazepine treatment in normal subjects (Palmieri et al., 1999). However, we would point out that all other effects of benzodiazepines on intracortical inhibition and cortical silent periods are the opposite to the findings of this study (Ziemann et al., 1996a; Palmieri et al., 1999), suggesting that cortical abnormalities in SPS occur despite the masking effects of benzodiazepines. The increased facilitation in the short interval paired paradigm cannot be explained by incomplete muscle relaxation in SPS patients, since voluntary contraction has the opposite effect of reducing facilitation at ISIs from 7 to 15 ms (Ridding et al., 1995; Chen et al., 1997), and in our own data with longer ISIs, contraction did not produce changes resembling those seen in SPS patients.

The finding of hyperexcitability of the motor cortex in SPS provides further evidence for a diffuse CNS pathology, as might be expected for a humoral autoimmune disorder. Nevertheless, the relationship of motor cortex hyperexcitability to the clinical manifestations of muscle stiffness and spasms remains obscure. The hyperexcitability of the motor cortex demonstrated in this study might produce an excessive corticospinal response upon activation, possibly contributing to stimulus induced muscle spasms. It would seem less likely to produce tonic activation of motor neurones thought to cause muscle stiffness at rest. Motor cortex hyperexcitability is also likely to affect descending control over spinal reflex circuits, and further studies will be needed to assess whether interactions between altered spinal and corticospinal inhibitory circuits or long-term adaptations produce the clinical manifestations of this disorder.

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References


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