Changes in excitability and impulse transmission following prolonged repetitive activity in normal subjects and patients with a focal nerve lesion

Matthew C. Kiernan, Ilona Mogyoros and David Burke

Summary
The present study was undertaken to document the excitability changes produced by prolonged high-frequency trains of impulses and to determine whether these changes in excitability would impair neural transmission in cutaneous afferents of patients with focal slowing of conduction across the carpal tunnel. A submaximal test stimulus was used to measure the changes in axonal excitability following trains of supramaximal stimuli delivered at 200 Hz for 30 s, 1 min or 2 min. These trains produced a prolonged depression in excitability in normal axons with gradual recovery to control levels over 20–30 min, presumably due to hyperpolarization associated with activation of the electrogenic Na\(^+\)/K\(^+\) pump. The decrease in excitability was demonstrable at nerve segments remote from the site of tetanic stimulation. Based on these findings, the effects on neural transmission were then assessed in normal subjects and patients using a supramaximal test stimulus following a 1-min tetanic train. In normal subjects there was a small activity-dependent decrease in amplitude of the compound sensory action potential (CSAP) associated with a prolongation in its latency. In patients with focal slowing of conduction across the carpal tunnel there was a more marked post-tetanic prolongation in latency, but the reduction in amplitude of the maximal CSAP was no greater than in the control subjects. It is concluded that activity-dependent conduction block is not a major cause of symptoms in carpal tunnel syndrome. It is suggested that the conduction slowing seen in patients with mild-moderate carpal tunnel syndrome could result from mechanisms other than demyelination.

Keywords: excitability; conduction; repetitive activity

Abbreviation: CSAP = compound sensory action potential

Introduction
Transmission of trains of impulses changes the excitability of axons and this can jeopardize conduction at sites of lowered safety margin (Bostock and Grafe, 1985). Conduction of brief trains of impulses activates nodal slow K\(^+\) conductances, producing a short-lasting subexcitability (Bergmans, 1970; Baker et al., 1987; Taylor et al., 1992; Bostock, 1995) that is not sufficient to produce conduction block in normal cutaneous afferents, presumably because of the normally high safety margin for impulse generation at each node (Miller et al., 1995). However, if the safety margin is impaired by, for example, a segment of demyelination, conduction block would be expected at that site (Bostock and Grafe, 1985). The focal conduction slowing in patients with carpal tunnel syndrome is often presumed to result from a focal disturbance to myelin and, indeed, paranodal demyelination has been documented pathologically (Ochoa and Marotte, 1973; Gilliatt, 1980; Brown, 1984). In a recent study, conduction of brief trains of impulses did not jeopardize conduction significantly in patients with focal conduction slowing due to carpal tunnel syndrome, even though it prolonged latency significantly (Miller et al., 1996). One reason for this finding may have been that the hyperpolarization produced by brief trains of impulses was not sufficient to impair impulse conduction despite a low safety margin.

Conduction of prolonged trains results in a more profound and long-lasting depression in axonal excitability due to activation of the electrogenic Na\(^+\)/K\(^+\) pump (Ritchie
conduction slowing was maximal over the carpal tunnel and antidromic conduction from palm to wrist, and antidromic conduction segment. To be included in the study, the amplitude of the median nerve measuring orthodromic median and ulnar potentials for digit IV. Palmar stimulation radial and median sensory potentials for digit I, and the digit II to wrist and elbow and from digit V to wrist, the establishment of focal slowing across the carpal tunnel (Table 1) (Goadsby and Burke, 1994). These studies included comparison of the digital sensory potentials from digit II to wrist and elbow and from digit V to wrist, the radial and median sensory potentials for digit I, and the median and ulnar potentials for digit IV. Palmar stimulation was performed for the median nerve measuring orthodromic conduction from palm to wrist and antidromic conduction from palm to digit II, in order to demonstrate that the conduction slowing was maximal over the carpal tunnel segment. To be included in the study, the amplitude of the CSAP from digit II had to exceed 5 μV to be accurately tracked by computer and, as a result, patients with very small or absent potentials were excluded.

The digital nerves of the index finger (digit II) were stimulated using ring electrodes around the proximal phalanx, and the evoked CSAP was recorded using bipolar self-adhesive surface electrodes 4 cm apart overlying the median nerve at the wrist. Skin temperature was measured at the second metacarpophalangeal joint and the wrist, and was kept >32°C at both sites by radiant heat and wrapping the limb in a blanket.

In the control subjects post-tetanic changes in axonal excitability were studied following conditioning trains of stimuli delivered at 200 Hz for 30 s, 1 min and 2 min. Each stimulus in the train was 0.1 ms in duration, set to be clearly supramaximal for the CSAP prior to the onset of tetanization. The test stimulus consisted of a single current pulse of 0.1 ms duration, delivered at 1 per second for at least 5 min prior to the onset of the tetanic stimulus train and then for 40 min after its end. The test stimulus was adjusted to produce a CSAP of 30–40% prior to tetanization, and excitability was measured using two different paradigms, the computer alternating between measuring the amplitude of the changing CSAP produced by a fixed stimulus (“amplitude tracking”), or altering stimulus intensity to keep the amplitude of the CSAP constant (“threshold tracking”; Bostock and Baker, 1988). The effects of the three high-frequency trains were studied in the same experiment (first 30 s, then 1 min, then 2 min) allowing time for full recovery as judged by complete return of both measures of excitability to control levels for >5 min before the next train was given. If there were residual effects of the preceding tetanic train they would have diminished the extent of recorded activity-dependent changes in excitability for the later trains.

Separate experiments were performed on the same 12 normal subjects to demonstrate that the excitability changes were not confined to the site of the tetanic stimulation. Trains of 1 min duration were delivered as usual to the index finger but the test stimulus was delivered in the palm, activating one of the digital nerves of the index finger.

The 12 normal subjects and 12 patients with carpal tunnel syndrome were then studied to determine whether the excitability changes produced by the tetanic stimulation at 200 Hz for 1 min impaired impulse conduction between the stimulating and recording electrodes. To eliminate the excitability changes at the site of stimulation, a supramaximal test stimulus was used, thus ensuring that a maximal nerve volley was set up regardless of local excitability changes. Both the test and conditioning stimuli were delivered to the digital nerves of the index finger using ring electrodes. The intensity of each stimulus in the 200-Hz train was supramaximal for the CSAP.

In all studies, CSAP amplitude was measured from the negative peak to the following positive peak (i.e. the falling phase of the potential). The latency of the CSAP produced by the test stimulus was measured to peak but these
Table 1  Sensory nerve conduction studies in the patient group and matched healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients</th>
<th>Significance</th>
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<tr>
<td></td>
<td>n</td>
<td>Age (years)</td>
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<tr>
<td></td>
<td>12</td>
<td>24-51</td>
<td>12</td>
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<tr>
<td>Sensory action potentials</td>
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<tr>
<td>Digit II: wrist amplitude</td>
<td>31.0±4.0 µV</td>
<td>16.9±7.5 µV</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Digit II: wrist conduction velocity</td>
<td>59.4±2.6 m s⁻¹</td>
<td>38.7±5.8 m s⁻¹</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Digit V: wrist amplitude</td>
<td>16.4±6.6 µV</td>
<td>16.6±4.9 µV</td>
<td>Not significant</td>
</tr>
<tr>
<td>Digit V: wrist conduction velocity</td>
<td>55.5±6.6 m s⁻¹</td>
<td>57.3±3.9 m s⁻¹</td>
<td>Not significant</td>
</tr>
<tr>
<td>Conduction delay</td>
<td>0</td>
<td>1.14±0.4 ms</td>
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Results are expressed as mean±SD.

measurements were limited by the sampling rate of the analogue to digital converter (10 kHz). In 10 studies (five patients and five controls), measurements of onset latency were also made using a cursor from analogue data on a storage oscilloscope. In the figures, data have been smoothed by averaging 10 consecutive points (i.e. resulting in one sample every 10-20 s).

Results

The sensory conduction studies for the 12 control subjects were within normal ranges for this laboratory (Goadsby and Burke, 1994), and are summarized in Table 1. In contrast, each of the 12 patients had evidence of focal conduction slowing across the carpal tunnel segment of the median nerve. In these patients comparison was made of the digital sensory potentials from digit II to wrist and elbow and from digit V to wrist, the radial and median sensory potentials for digit I, and the median and ulnar potentials for digit IV. Using the ulnar and radial values, a conduction time was calculated for the digit II-wrist conduction distance and, by subtracting this value from the measured latency, the delay in conduction across the carpal tunnel segment was estimated. The calculated degree of slowing across the carpal tunnel was 1.14±0.4 ms (mean±SD). The above tests and those of palmar stimulation (Goadsby and Burke, 1994) established that conduction was normal in the median nerve proximal and distal to the carpal tunnel segment.

Changes in excitability produced by prolonged trains of impulses

Conduction of impulses at 200 Hz for 30 s depressed the excitability of digital afferents in normal subjects (Fig. 1). These excitability changes were measured using two methods simultaneously: tracking the changes in amplitude of the CSAP produced by a constant submaximal stimulus, and tracking the changes in stimulus intensity required to produce a CSAP of constant amplitude. The post-tetanic decrease in excitability was maximal immediately after cessation of the conditioning train, CSAP amplitude then being 36.8% of control (Fig. 1A) and the current required to generate the target potential increasing to 122% of the control level (Fig. 1B). These changes in amplitude and threshold returned gradually to control values over some 20-30 min.

The post-tetanic subexcitability was more profound following tetanic stimulation for 1 min (Fig. 2). The greater magnitude of the subexcitability was manifested more by slower recovery than by greater depth. With the two
test paradigms, amplitude fell to 38.3% of its control level (Fig. 2A), and the threshold current required to generate the potential increased to 132.7% (Fig. 2B), both recovering gradually to baseline. Increasing the duration of conditioning train to 2 min had little effect on the depth of the subexcitability but distorted the recovery. There was a reduction in the amplitude of the test potential to 40.8% (Fig. 3A) and an increase in threshold to 147% (Fig. 3B) of their control values. However, with this train duration, there was an inflection on the recovery curves for both amplitude and threshold, with an early peak at 3–4 min and a plateau phase for the next 5 min. The greater hyperpolarization expected due to the greater impulse load was best seen ~10 min after the train. With longer tetanic trains, this inflection becomes more prominent and, with trains of 5–10 min duration, there may be an overt but transient phase of hyperexcitability, during which subjects experience paraesthesiae (Ng et al., 1987; Applegate and Burke, 1989; Macefield and Burke, 1991; see also Bostock and Bergmans, 1994).

These changes in excitability were accompanied by an increase in latency of the constant-amplitude CSAP of the threshold-tracking paradigm, presumably because the activity-dependent hyperpolarization resulted in delayed impulse initiation at each node (Fig. 4). Following conditioning trains of 30 s duration, there was a maximal prolongation in latency to negative peak of 0.12 ms, with return to control levels by 16 min. With longer conditioning trains of 1-min duration, the latency increased by 0.17 ms and recovered more slowly. With 2-min conditioning trains, the changes in latency were greater, confirming that the extent of the latency increase was dependent on impulse load.

**Is activity-dependent subexcitability localized to the site of stimulation?**

The above studies established that the 1-min conditioning train produced near maximal subexcitability, not obviously complicated by the short-lasting processes responsible for the transient increase in excitability that can follow long tetanic trains (Applegate and Burke, 1989; Macefield and Burke, 1991), as occurred with 2-min trains. However, before investigating whether prolonged trains of impulses delivered to digit II produce activity-dependent conduction block at the site of nerve pathology in carpal tunnel syndrome, it was necessary to confirm that the post-tetanic subexcitability occurred at nodes along the course of the nerve; i.e. that the excitability changes were not restricted to the site of tetanic stimulation.

When studied in the same 12 subjects, conditioning trains
of 1-min duration delivered to the index finger depressed the excitability of afferents in the palm (Fig. 5), presumably an effect that was present throughout the entire length of the stimulated axons. The reduction in amplitude of the test potential was less than that when the tetanic train and the test stimulus were both delivered to the index finger. However, the smaller magnitude of changes seen with test stimulation in the palm may be because some axons contributing to the CSAP were not tetanized: the test volley may have involved some axons from intrinsic muscles or from digital nerves other than those that were tetanized. Nevertheless, if the activity-dependent depression in excitability were sufficient to affect impulse conduction, it should do so with the time course illustrated by the filled circles in Fig. 5.

**The effect of activity on impulse transmission**

The effects on impulse transmission were measured using the changes produced by 1-min tetanic trains in maximal CSAPs. By using a supramaximal test stimulus, the effects of local excitability changes at the site of stimulation were eliminated such that a maximal sensory volley was set up. A reduction in the size of the test potential would then be attributable to impaired impulse transmission in axons between the stimulating and recording sites due to dispersion of the afferent volley or conduction block in some axons.

**Latency of the maximal test potential**

Regardless of whether there was transmission failure, a change in latency of the maximal test potential would be expected because, in conducting axons, the activity-dependent hyperpolarization should result in later impulse initiation at each node. A post-tetanic increase in the latency to the peak of the CSAP was seen in both the patients and the controls (Fig. 6A). The maximal increase in peak latency occurred immediately after the end of the tetanic train and, in normal subjects, was $0.18 \pm 0.09$ ms (mean±SEM). The latency change was larger in patients with carpal tunnel syndrome, maximally $0.38 \pm 0.04$ ms (mean±SEM), but in both, recovery followed a similar time course to that seen with submaximal test potentials in normal subjects (Fig. 4).

As mentioned in Methods, the accuracy of these latency measurements was limited by the sampling rate of the computer (10 kHz). To confirm the changes, latency to the onset of the CSAP was measured from analogue traces every minute following the 1-min tetanic train in five control subjects and five patients (Fig. 6B). In control subjects, latency increased by $0.19 \pm 0.06$ ms (mean±SEM). Again, the change in onset latency was greater in patients of $0.27 \pm 0.15$ ms (mean±SEM), and recovery followed a similar time course as latency to peak.

The above latency changes and their time course establish that the tetanic stimulus trains had the expected effects on the test potential of both patients and controls and were no less effective in the patients. (Indeed, the increases in latency...
Fig. 6 The changes in latency of the supramaximal test potential after tetanic stimulation at 200 Hz for 1 min. In A, latency is measured to peak in 12 patients (closed circles) and 12 controls (open circles). In B, latency is measured to onset from analogue traces in five patients (closed circles) and five controls (open circles). Results are expressed as mean ±SEM.

were consistently greater in the patients, though so too were the control latencies.) There was no correlation between the average latency increase in the patients during the first minute after the end of the tetanic train and the pre-existing conduction delay across the carpal tunnel.

Amplitude of the maximal test potential
The changes in amplitude of the maximal CSAP were small, but demonstrable in all control subjects. The maximal reduction in amplitude of the test potential occurred immediately after the end of the tetanic stimulus train, to 93% of the control amplitude, with subsequent slow recovery, that was complete by 10 min (Fig. 7). Averaging the data over the first minute following the end of the tetanus, the test potential was reduced to 94.6% of the control. In the patients, the mean amplitude of the test potential immediately after the end of the tetanic train was 90.1%, less than in normal subjects but within the range created by the normal mean ±2 SDs (Fig. 8). The average test potential over the first minute following the end of the tetanus was 96.2% of control, greater than in normal subjects. The data for individual patients had considerable scatter about the mean (Fig. 8A), greater in the patients (SD 5.5%) than in the normal subjects (SD 4.4%). This was the result of greater noise on a CSAP of lower amplitude than in controls (see Table 1) and possibly difficulty in relaxing completely during and after tetanic stimulation.

To determine whether the scatter of data points concealed abnormal responses in the patients, the data for individual patients were examined to see if values for an individual consistently fell below the control mean value minus 2 SD. The amplitude of the test potential for only two patients fell below 2 SD of the control mean for three consecutive measurements during the first 10 min following the end of the tetanic train. These data points occurred immediately after the end of the tetanic train, and in the next minute the data for the two patients averaged 87.2% and 93.6% of control. The pre-existing conduction delay across the carpal tunnel segment in the two patients was 0.78 and 2.02 ms, compared with the mean delay of 1.14 ms. Importantly, the 10 patients in whom there was no evidence of activity-dependent conduction block had a mean delay across the carpal-tunnel segment of 1.09 ms. For the group of 12 patients, there was no relationship between the average reduction in amplitude during the first minute after the end of the train and the delay across the carpal tunnel segment.

Discussion
The present study establishes that conduction of prolonged trains of impulses decreases the excitability of normal human cutaneous afferents along the course of the nerve, not just at the site of stimulation. The effects of this subexcitability on impulse conduction were studied in normal subjects and in patients with carpal tunnel syndrome. While changes in excitability had clear effects on the amplitude and latency of the CSAP, the decrease in amplitude was small in the patients, on average, no greater than in control subjects. Activity-dependent conduction block may play little role in the symptoms of patients with carpal tunnel syndrome.
Activity-dependent hyperpolarization and conduction block

When normal axons conduct a train of impulses, the axonal membrane undergoes an activity-dependent depression in excitability, associated with two positive after-potentials (Gasser, 1935), which appear to have different mechanisms dependent on the impulse load. With brief trains, it is due chiefly to activation of a nodal slow K⁺ conductance (Baker et al., 1987; Taylor et al., 1992; Miller et al., 1995; Bostock, 1995). With longer trains, the mechanism is probably activation of the electrogenic Na⁺/K⁺ pump (Bergmans, 1970, 1982; Schoepfle and Katholi, 1973; Raymond, 1979; Bostock and Grafe, 1985; Applegate and Burke, 1989; Gordon et al., 1990; Morita et al., 1993; Bostock and Bergmans, 1994), although a contribution from a Na⁺-activated K⁺ conductance is also possible, even likely (Koh et al., 1994). The pump exchanges two (extra-axonal) K⁺ ions for three (intra-axonal) Na⁺ ions, and this results in hyperpolarization, due to a net deficit of positive ions on the inner aspect of the membrane. As expected from animal experiments (Morita et al., 1993; see also Bergmans, 1970; Bostock and Bergmans, 1994), the depth of the post-tetanic subexcitability reached a maximum, and the greater impulse load associated with longer trains then affected the time course of recovery. Accordingly, it is likely that the 1-min trains used in the present study would have resulted in the maximal hyperpolarization possible through this mechanism.

Bostock and Grafe (1985) showed that rat nerve fibres with a reduced safety margin for conduction, due to focal demyelination, were blocked by conduction of trains of impulses. They found that conduction block was due to membrane hyperpolarization, which could be compensated by applying a depolarizing current or prevented by replacing Na⁺ in the extracellular solution by Li⁺. From this they concluded that the cause of conduction block in demyelinated fibres conducting trains of impulses was due to membrane hyperpolarization resulting from electrogenic sodium pumping. Further confirmation was provided by Kaji and Sumner (1989) who demonstrated that inhibition of the Na⁺/K⁺ pump by the cardiac glycoside ouabain, reversed rate-dependent conduction block in demyelinated rat axons, suggesting a possible therapeutic use for such agents in multiple sclerosis and other demyelinating disorders.

The present study addresses issues raised in a previous paper by Miller et al. (1996), who studied the effects of brief conditioning trains on impulse conduction in patients with carpal tunnel syndrome. In that study, trains of 10 stimuli at 200 Hz produced no evidence of activity-dependent conduction block, even though they depressed excitability with a time course typical of that expected with activation of slow K⁺ conductances. In the present study, a greater stress was used, producing the more profound long-lasting hyperpolarization associated with activation of the Na⁺/K⁺ pump, but again there was little evidence of activity-dependent conduction block in the majority of patients, even though all had a focal conduction delay across the carpal tunnel. While it is possible that some axons were blocked in all 12 patients, insufficient were so affected to produce detectable changes in the CSAP in the 10 of the 12 and, importantly, the amplitude depression was not greater in the patients than the normal subjects. Accordingly it can be concluded that the pathologically slow axons of patients with carpal tunnel syndrome do not behave as would be expected for demyelinated axons with a marginal safety factor.

Activity-dependent changes in latency

Prolonged tetanization resulted in a prolongation in latency of the test potential, presumably due to a slight delay in action potential generation at each node produced by the activity-dependent hyperpolarization. This latency increase was greater in patients than control subjects. From the study of Miller et al. (1996) one might then have expected a greater reduction in amplitude to be seen in the patients, on the basis of...
Pathophysiology of carpal tunnel syndrome

Before drawing conclusions about the pathophysiology of carpal tunnel syndrome from the current study, it must be remembered that studies were performed on patients with a CSAP >5 μV. Accordingly, it is likely that only patients with moderate carpal tunnel syndrome were studied. However, all patients had evidence of focal conduction slowing and in these patients, there was no convincing evidence for greater activity-dependent conduction block than in normal subjects. Such evidence as there was occurred in only two of 12 patients and did not parallel the expected time course of the post-tetanic decrease in excitability.

The present study does not exclude the possibility that repetitive stimulation produced activity-dependent conduction block in healthy control subjects, though we favour the view that in these subjects the post-tetanic amplitude attenuation was probably due to temporal dispersion of the compound volley. Whether or not such prolonged high-frequency repetitive activity produces conduction block in afferents with a normal safety margin, it is still safe to conclude that the focal slowing of carpal tunnel syndrome is not associated with increased sensitivity to the effects of repetitive activity. This suggests that activity-dependent conduction block plays little role in the symptoms of patients with carpal tunnel syndrome of mild-moderate severity.

The low amplitude CSAPs of the patients (Table 1) could have been due to axonal loss, conduction block or dispersion of the afferent volley. If conduction block was a significant factor, it would be reasonable to expect some conducting axons to have a critically impaired safety margin, and impulse conduction to fail in these axons when they were subjected to tetanization. The failure to demonstrate activity-dependent conduction block could be explained by a paucity of such axons, but it would be reasonable to question the underlying premise of the study, namely that the conduction slowing results from focal demyelination. An alternative explanation for conduction slowing would be inactivation of Na⁺ channels due to membrane depolarization, perhaps analogous to that occurring during acute ischaemic/compressive lesions (see Bostock et al., 1994; Miller et al., 1996). Paradoxically, the post-tetanic hyperpolarization would then not impair impulse conduction: indeed it might improve the impaired safety margin associated with axonal depolarization.

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