Time course of action potentials recorded from single human afferents

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SUMMARY

It was the object of this study to measure the time course of the action potential in individual human sensory nerve fibres in relation to conduction properties of the axons. For this purpose, the technique of percutaneous microneurography was combined with intradermal electrical stimulation of distal portions of the axons. Recordings were made at the wrist level from 57 type-identified mechanoreceptive median nerve afferents [mainly rapidly adapting (RA) and slowly adapting type I (SAI)] innervating the glabrous skin of the hand. Measurements were made of the duration and time-to-peak of the positive peak of the diphasic (large positive phase followed by smaller, slower negative phase) action potential typically recorded using microneurography. Durations ranged from 0.31 to 0.75 ms (mean 0.50 ms) and times-to-peak from 0.12 to 0.45 ms (mean 0.21 ms), with no difference between afferent categories (RA, SAI). Time-to-peak was strongly positively correlated with duration (linear $r = 0.81$). Conduction velocity was measured over the distance extending from the point of intradermal stimulation (typically in the fingertips) to the point of recording at the wrist (distal conduction velocity). Absolute refractory period was measured using paired stimuli applied at the point of intradermal stimulation, within the receptive field of the afferent (distal absolute refractory period). Distal conduction velocities ranged from 15 to 60 m/s (mean 33 m/s), and distal refractory periods from 0.7 to 4.5 ms (mean 2.1 ms), with no difference between afferent types (RA, SAI). Distal absolute refractory period was inversely correlated with distal conduction velocity. The data were slightly better described assuming a non-linear (exponential) relationship; the non-linear correlation coefficient was $-0.77$.

The time course of the action potential varied inversely with distal conduction velocity and directly with distal absolute refractory period. The time-to-peak versus conduction velocity data were slightly better described by a power than a linear relationship. Coefficients of correlation were: duration versus conduction velocity, linear $r = -0.76$; time-to-peak versus conduction velocity, non-linear $r = -0.64$; duration versus absolute refractory period, $r = 0.70$; time-to-peak versus absolute refractory period, $r = 0.76$. Extensive intercorrelation between the variables duration, distal conduction velocity and absolute refractory period was revealed by multiple correlation techniques. Inter- and intra-subject skin temperature variation was within 5°C. Correcting the time course, conduction velocity and absolute refractory period values for temperature variation within this limited range did not affect the results.

The results demonstrate that the time course of the action potential varies systematically...
with distal axon conduction properties, in normal human subjects. The present approach for study of action potential time course and conduction properties of single axons in man promises to provide a sensitive means for assessing the impact of nerve disease on neural impulse transmission.

**INTRODUCTION**

The time course of the propagated action potential reflects the time course of the underlying local current spread, capacitative and ionic currents (Hodgkin and Huxley, 1952). Sufficient is known about the events underlying action potentials, and factors that influence them, to permit reconstruction of a propagated action potential, with good correspondence to experimental data, for both unmyelinated (Hodgkin and Huxley, 1952) and myelinated (Goldman and Albus, 1968, using equations from Frankenhaeuser and Huxley, 1964) fibres.

Further theoretical treatments (Rushton, 1951; FitzHugh, 1973) have predicted that the time course of an action potential will be constant for axons of different size if (i) membrane properties of axons do not vary with fibre size and (ii) axons are dimensionally similar (internode length, myelin thickness and nodal area are proportional to fibre diameter). Early observations seemed to validate this: Gasser and Grundfest (1939) concluded that spike duration was invariant for the full range of medullated A fibres (diameters of \( \sim 2-16 \mu m \) in the cat; Gasser and Grundfest, 1939). However, later measurements made from individual fibres conducting over a wide range of velocities demonstrated an inverse relationship of action potential duration, rise time and fall time to conduction velocity (Paintal, 1966, 1967). Arguing that dimensional similarity holds reasonably well for normal peripheral nerves, Jack (1975) has taken the finding of systematic variation of action potential time course as indicative of systematic variation with fibre size of a specific membrane property or properties.

The time course of the action potential in individual human nerve fibres, and its relationship to fibre size, have not yet been described. Until recently, study of individual nerve fibres in man has remained essentially inaccessible: it has been necessary to generalize from animal studies. However, the introduction of the technique of percutaneous microneurography (Vallbo and Hagbarth, 1968) has permitted direct study of single axons in man, including study of conduction properties such as conduction velocity (Johansson and Vallbo, 1983; Mackel, 1988) and refractory period (Mackel, 1988).

In the present work, use of microneurography is extended to the study of the time course of action potentials recorded from individual human mechanoreceptive afferents. The possibility of systematic variation of time course with the conduction properties of distal conduction velocity and distal refractory period of the axons is examined. The data will be used for comparison with data from nerve disease, where action potential electrogenesis and propagation may be altered (McDonald, 1963; Bostock and Sears, 1978; Brismar, 1983; see also Waxman, 1978).

**METHODS**

Axonal conduction properties and time course of the action potential were measured for mechanoreceptive afferents innervating the glabrous skin of the hand, using the technique of percutaneous microneurography combined with intradermal electrical stimulation of distal portions of the axons (Mackel, 1988). Twenty-three experiments were performed on 12 healthy volunteers (aged 22–36 years). Recordings were obtained
from median nerve afferents at the wrist level. The study was approved by the Ethical and Human Rights Commission of New York Hospital—Cornell University Medical Center (protocol number 0787-923) and all examinations were performed with the written informed consent of the subjects and according to the guidelines of the Declaration of Helsinki.

**Recording and stimulation procedures**

Single unit activity was recorded from type identified (see below) cutaneous afferents, using tungsten electrodes (Vallbo et al., 1979) coated with parylene (Schmidt, 1983). The electrodes had a tip diameter of 3–5 μm and impedances between 100 and 500 kΩ. The electrode impedance (at 1000 Hz) was measured *in situ* during the recording sessions. Recording was monopolar versus an indifferent tungsten electrode inserted under the skin, 2–3 cm proximal to the recording site. The neural signals were amplified via a battery-powered preamplifier (A 1-B, BAK Electronics, Maryland, USA), filtered (low pass 6 kHz, high pass 1–100 Hz, and see below) and fed through a linear isolation unit (BSI-1, BAK Electronics, Maryland, USA).

The nerve signals were displayed on an oscilloscope and audiomonitored. Signals were also displayed and averaged (typically 10 sweeps) on a digital storage oscilloscope (Tektronix R 468); experimental measurements were made using the cursors of the instrument. [As illustrated previously, averaging smoothed the records without changing, within the scope of the present measurements, the latency, amplitude or shape of the positive phase of the action potential (Mackel, 1988).] Records were photographed directly from the storage oscilloscope (Tektronix camera).

Measurements were made from afferents which were first type identified as RA (rapidly adapting, recently also called FAI, for fast adapting type I, by Vallbo and Johansson, 1984), PC (Pacinian afferents, recently also called FAII, for fast adapting type II), SAI and SAII (slowly adapting types I and II), according to the criteria of the discharge characteristics in response to mechanical skin indentations and receptive field properties (Vallbo et al., 1979), as described previously (Mackel, 1988). Rapidly adapting type units are believed to innervate Meissner end organs, SAI units Merkel cells, SAI units Ruffini endings and PC units Pacinian corpuscles (Vallbo and Johansson, 1984). The most sensitive spot(s) within a receptive field were localized (under magnification, ×5) with calibrated von Frey hairs (Stoelting Company, Chicago, USA) and marked with ink.

After identification of an afferent and localization of its receptive field, the tip of a bipolar concentric needle electrode was inserted intradermally into the most sensitive spot of the receptive field. The procedure of intradermal stimulation, the tests for confirming identity of the units and the stimulus parameters used for conduction velocity and refractory period measurements are described in detail elsewhere (Torebjörk and Hallin, 1974; Mackel, 1988; Torebjörk and Ochoa, 1990). Briefly, bipolar rectangular pulses of 50 μs duration were delivered at a frequency of 2/s to distal portions of the axons. During measurements, stimulus strength was set at twice electrical threshold. Previous work indicated that, under these conditions, current spread is limited (to within 2–3 mm of the point of lowest mechanical threshold in the receptive field) and the stimulation does not become unpleasant or painful to the subjects (Mackel, 1988). Conduction velocities were determined from latencies of the orthodromically conducted action potentials and the conduction distances. Latencies were measured from the electrical stimulus artefact to the beginning of the response (see, e.g., Fig. 1A). Conduction distances were measured by aligning a thread along the approximate course of the median nerve, from the point of stimulation to the recording site at the wrist. Absolute refractory periods were determined using paired electrical stimuli of identical intensity and duration, and variable interpulse interval (Mackel, 1988). Absolute refractory period was taken as the shortest interpulse interval at which an action potential was generated and propagated in response to the second stimulus, consistent with the original definition by Adrian (1921). Previous work indicates that using stimuli of two times threshold leads to slightly longer measures of the absolute refractory periods (by ~10%) than obtained when using stronger stimuli (Mackel, 1988). The painful or uncomfortable sensations produced by repeated stronger stimuli, however, precludes their routine usage.

**Measurement of the time course of the action potential**

Action potentials recorded from single human mechanoreceptive nerve fibres are typically diphasic, consisting of a positive phase followed by a smaller, slower negative phase (Torebjörk et al., 1970; Vallbo, 1976; and see Fig. 1). Interpreted in light of experimental work (Tasaki, 1952), such action potentials are presumably recorded when the electrode tip impales the myelin, creating a low impedance path to the inside of the fibre (Vallbo, 1976). Negative potentials (Fig. 1d), resembling typical triphasic extra-axonal recordings
Fig. 1. Shapes of action potentials recorded microneurographically (A–F) and influence of high pass filter settings on action potential waveforms (G–I). Action potentials are typically positive-negative (A, B, C, E, F) and may have a deflection on the rising (B) or falling (C) phase of the positivity. Occasionally, pure negativity (D) is recorded. Measures of action potential time course were made on a fast time base, from strictly positive going signals (E, F). Durations were measured from the point of the upstroke from baseline to the point where the potential crossed the baseline (arrows in E and F), regardless of whether the following negativity returned to baseline (E) or not (F). Times-to-peak were measured to maximum positivity (not indicated). The shapes of action potentials were superimposable at high pass filter settings from 1 to 100 Hz (G, left; H, I, left). At 300 Hz, the falling phases were occasionally faster (compare I, middle with G, right), at 500 Hz always faster (I, right). The numbers next to or underneath the traces in G–I indicate the high pass filter settings. All recordings are averages of 10 sweeps and were photographed off the oscilloscope. In G–I two traces (recorded at different filter settings) are superimposed. Time base in B–D, same as in A.

(Murakami et al., 1961) are obtained more rarely. The diphasic, mainly positive action potentials may show a notch or second peak on the positive phase (Fig. 1B, C), presumably reflecting development of a conduction block at the recording site (Tasaki, 1952; Vallbo, 1976). Measurements of time course were made only from diphasic, mainly positive, action potentials that did not display such a notch or second peak.

Duration was measured for the positive phase only of the action potential, from the point of the upstroke from the baseline to the point where the potential crossed the baseline, as illustrated in Fig. 1E, F. Time-to-peak of the positive phase was measured to the point of maximal positivity. Because the return of the negative phase to baseline often proved difficult to determine, measurements of the duration of the diphasic spike were not collected routinely. Measurements were made from the averaged records displayed on a fast time base (0.5 or 1 ms/division) of the digital storage oscilloscope. Measurements were made to the second decimal place, using the cursors of the instrument. All measurements were made twice.

The effect of signal filtering on the shape of the action potential, and therefore on measurements of time course, was examined for 11 afferents in four subjects. High pass filter settings were varied from 1 to 500 Hz (1, 10, 50, 100, 300, 500 Hz); the low pass filter remained at 6 Hz. Stable recordings were obtained from seven afferents; examples from three of these are illustrated in Fig. 1G, I. As seen in the figure, varying the filter settings from 1 to 100 Hz does not affect the shape of the positive phase of the action potential: the potentials are superimposable. Distortion is sometimes apparent with filter settings at 300 Hz (compare Fig. 1I with 1G) and clear at 500 Hz: the fall-off of the positive potential is faster, which would lead to shorter measures of duration. As anticipated, the slower negative phase of the action potential was truncated with all but the lowest filter settings (Fig. 1H). For the remaining four afferents, shifts occurred in notches of the action potentials. Nonetheless, similar observations were obtained: except for the shift in the notch, the positive phases of action potentials recorded with filters of 1–100 Hz were superimposable. These observations indicated that high pass filtering at 100 Hz would not distort measurements of the duration and time to peak of the positive phase of the action potential. Since filtering at 100 Hz improved recording by
limiting common baseline fluctuations [line noise (60 Hz), breathing movements, arterial pulsations], most measurements were made at this setting.

Temperature measurements

The experiments were conducted at room temperature (24–25°C). Since temperature influences the experimental measures under study (e.g. Buchthal and Rosenfalck, 1966; Paintal, 1966; see also Results), skin temperature was monitored using a surface thermoprobe (Sensortek, model BAT-12) attached to the proximal part of a digit. Skin temperature varied from 30 to 35°C between subjects. Intra-subject variation over a 2 h experimental session did not exceed 2°C within the 30–35°C range, with temperatures rising, falling or both during the session.

Because the experimental measures were made at different sites in the hand (refractory periods from stimulating distal portions of the axons, typically in the distal fingers; action potential time course measured more proximally at the site of recording at the wrist), the measurements might be differentially affected by local variations in temperature. To examine this possibility, local temperature variations were studied in six subjects. Surface temperature was measured from the fingertip, the proximal part of the digit, and from the wrist. Additionally, a needle probe was inserted subcutaneously to measure temperature near the nerve, at the proximal part of the digit and at the wrist. As expected, the fingertips tended to be cooler than more proximal parts, with the difference in temperature between fingertip and wrist ranging from 0 to 2°C. Most of the temperature differential occurred between fingertip and proximal part of the digit, ranging from 0 to 1.5°C and averaging 0.55°C (±0.7°C, SD, n = 6). The temperature differential between proximal digit and wrist averaged 0.3°C. As reported previously (Kunesch et al., 1987; Mackel, 1988), temperatures measured near the nerve tended to be somewhat higher than surface temperatures, by up to 1°C at both the proximal digit and the wrist. The average temperature differential between surface and near nerve was 0.4°C [at both sites; in present and previous data (Mackel, 1988) combined: n = 15, SD = 0.5°C].

For the purposes of correcting the temperature (see Results), the following simplifications were made. (i) Temperatures at the wrist and proximal digit were considered to be the same. Adjustments of time course and conduction velocity were made using these values. (ii) Temperature at the fingertips was considered to be less, by 0.5°C, than that at the proximal digit. Refractory periods were adjusted using this value. (iii) Near nerve temperatures were estimated by adding 0.5°C to surface temperature. (iv) Data were standardized to a near nerve temperature of 32.5°C: 32°C being the average surface temperature at the proximal digit of normal subjects in the present and previous (Mackel, 1988) work (X±SD = 32.2°C±1.6, n = 19), and 0.5°C allowed for the difference between surface and near nerve.

Data analysis

For the most part, data were tested for simple linear correlation (Hays, 1969; Zar, 1984) between pairs of variables (absolute refractory period, conduction velocity, duration, time-to-peak). The correlation coefficient, r, and the coefficient of determination, r^2, were calculated. The value r is an index of the direction and degree of the association between variables; r^2 indicates the proportion of variance in one variable that can be accounted for by its relationship to the second variable. The absolute value of r, |r|, is also equivalent to the degree of association between the actual values of one variable and the values predicted from its relationship (i.e. the regression equation) to the second variable. In this conception, the goodness of the assumed underlying relationship is emphasized.

In a few cases, the assumption of an underlying linear relationship appeared inappropriate from the scatter plots of the data. The data were re-tested for correlation assuming underlying exponential or power relationships, as judged from the plots. This was done by taking the logarithm of one (for exponential) or of both (for power) variables and applying linear correlation techniques to the transformed data (Zar, 1984). The procedure yielding the best r and r^2 was used to describe the data, where 'best' indicates an increase in r^2 (amount of variance accounted for) by ≥ 10%. In effect, values of r and r^2 for exponential and power correlation were identical or nearly so, and only one set of values is presented. Furthermore, for the restricted ranges of the data obtained, the improvements in r and r^2 with non-linear correlation were modest.

Since several variables were examined for many of the afferents studied, multiple linear correlation procedures were also used when warranted. In particular, coefficients of partial correlation were calculated to determine the amount of correlation persisting between two variables when covariation due to a third variable was removed (Hays, 1969; Zar, 1984). Additionally, the multiple correlation coefficient, R, and multiple coefficient of determination, R^2, were calculated (Hays, 1969). The value R^2 has the same sense as r^2, extended to three or more variables. The value R is non-directional, having the meaning of the absolute value of r.
RESULTS

Data were obtained from 57 type-identified afferents innervating the glabrous skin of the hand: 33 RA, 19 SAI, four SAII and one SA unit not further identified. The sample under-represents SAII and PC units (compared with Johansson and Vallbo, 1979). More SAII and PC units were encountered, but could not be studied because of the difficulty, and discomfort to the subject, in attempting to position the intradermal electrode and to stimulate near the afferent end organs. The greater difficulty with SAII and PC afferents than with RA and SAI afferents probably arises, in part, from the deeper location of their end organs (deep dermal and subcutaneous positions, Miller et al., 1958) compared with those of RA and SAI afferents [underside of epidermis (Miller et al., 1958; Quilliam and Ridley, 1971)]. The proportion of RA to SAI afferents is typical of previous samples (Johansson and Vallbo, 1979; Mackel, 1988). Not all measurements were obtained for all units: full sets of data, including conduction velocity, absolute refractory period and duration and time-to-peak of the positive phase of the action potential, were obtained for 34 units. As demonstrated in the final section of the Results, correcting for temperature variation within the limited range of temperatures observed for the present set of normal subjects did not affect the results. Therefore, unless otherwise indicated, data are presented uncorrected for temperature.

Time course of the action potential

As illustrated in Fig. 2 and summarized in Table 1, durations of the positive phase of action potentials recorded at the wrist level ranged from 0.31 to 0.75 ms, with a mean of 0.50 ms, for all units (n = 46). From this figure and table it is apparent that there

![Figure 2](image-url)
TABLE 1. SUMMARY OF DATA

<table>
<thead>
<tr>
<th></th>
<th>CV</th>
<th>ARP</th>
<th>Duration</th>
<th>Time-to-peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapidly adapting</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>32.7 m/s</td>
<td>±10.1 m/s</td>
<td>33</td>
<td>2.2 ms</td>
</tr>
<tr>
<td>Slowly adapting type I</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
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<tr>
<td></td>
<td>35.9 m/s</td>
<td>±9.0 m/s</td>
<td>19</td>
<td>1.9 ms</td>
</tr>
<tr>
<td>All</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>33.4 m/s</td>
<td>±9.6 m/s</td>
<td>57</td>
<td>2.1 ms</td>
</tr>
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</table>

CV = conduction velocity; ARP = absolute refractory period; SD = standard deviation; N = number.

are no differences in duration associated with type of afferent: means and standard deviations were comparable for the two largest subsamples studied [RA and SAI units: 0.2 < P < 0.5, two-tailed t test (Hays, 1969)]. The sample of SAII afferents is too small for valid comparison; however, the values obtained are within range of values for RA and SAI afferents (Fig. 2). Times-to-peak of the positive phase ranged from 0.12 to 0.45 ms, averaging 0.21 ms, for all units, with no difference between subsamples (Fig. 2; Table 1).

As illustrated in Fig. 2, time-to-peak was positively correlated with duration: longer-lasting action potentials had longer times-to-peak. The value of the linear correlation coefficient (r) was 0.81 (n = 44), and highly significant compared with the null hypothesis of no correlation (P < 0.001, two-tailed t test, Zar, 1984). This high level of correlation indicates that more than half of the total variability (r² = 0.66) in time-to-peak is accounted for by linearly related variability in duration (or vice versa).

Conduction velocities and absolute refractory periods

Conduction velocities and absolute refractory periods for different types of mechanosensitive afferents are illustrated in Fig. 3 and summarized in Table 1. Conduction velocities for propagation to the wrist level ranged between 15 and 60 m/s for all afferents (n = 57), averaging 33 m/s, with no apparent differences between types (i.e. RA or SAI) of mechanosensitive afferents. This is consistent with results obtained in an earlier study, where the overall mean and standard deviation were 36 ± 10 m/s (Mackel, 1988).

Absolute refractory periods of distal portions of the axons (see Discussion) ranged from 0.7 to 4.5 ms for all afferents (n = 47), with a mean and standard deviation of 2.1 ± 0.9 ms, consistent with previous results (mean ± SD: 2.1 ± 0.8 ms; Mackel, 1988). As in the previous study (Mackel, 1988), there was no evidence for difference in the duration of the absolute refractory period associated with type (RA or SAI) of afferent (Fig. 3; Table 1).

Figure 3 illustrates the inverse correlation between distal conduction velocity and absolute refractory period of the distal portions of the axon: slowly conducting fibres had longer refractory periods. Data in Fig. 3a are from the present experiments, with different symbols representing different types of afferents and illustrating that the inverse relationship holds for the different types of afferents. The data appear to become non-linear as the (lower) limits for conduction velocity and absolute refractory period are approached, and
non-linear (exponential) correlation procedures yielded $r$ and $r^2$ values of $-0.77$ and $0.59$, respectively ($n = 47$, all afferent types). These values are slight improvements over the linear values ($r = -0.71$, $r^2 = 0.50$) according to the criterion adopted, and are highly significant [$F(1,45) = 65.22; P < 0.005$ (Zar, 1984)] compared with the null hypothesis of no relationship between conduction velocity and absolute refractory period ($r^2 = 0$ versus $r^2 > 0$).

In Fig. 3B, the current data on conduction velocity and absolute refractory period are presented together with previous data (Mackel, 1988) to illustrate the consistency between samples. For the combined data ($n = 77$), non-linear (exponential) correlation procedures gave $r$ and $r^2$ values of $-0.74$ and $0.55$, respectively (slightly better than the linear values of $r = -0.70$, $r^2 = 0.49$).

Correlation of time course of the action potential with conduction properties of the axon

Figure 4 presents the data on time course of the action potential plotted against distal conduction velocity (Fig. 4A, B) and against distal refractory period (Fig. 4C, D). As illustrated in Figs. 4A, B, duration of the positive phase and time-to-peak decreased as speed of propagation of the action potentials increased. For duration versus conduction velocity, the linear correlation coefficient was $-0.76$ ($n = 46$, all afferent types) and highly significant [$P < 0.001$, two-tailed $t$ test (Hays, 1969; Zar, 1984)]. For the time-
to-peak versus conduction velocity data, non-linear (power) correlation procedures yielded $r$ and $r^2$ values of -0.64 and 0.41, respectively ($n = 44$, all afferent types), slightly better than the values for linear correlation ($r = -0.57$; $r^2 = 0.32$), and highly significant [$F(1,41) = 28; P \ll 0.0005$ (Zar, 1984)]. The regression coefficient for the power relationship was $-0.67$.

Contrary to the conclusion of Paintal (1966), it was not necessary for measurements of the time course to be carried out to the second decimal place for the correlations to appear. With measurements rounded to the nearest tenth, the linear correlation coefficient for duration versus conduction velocity remained virtually the same, at $-0.79$. For
time-to-peak versus conduction velocity, the coefficient for non-linear (power) correlation lessened to $-0.50$, but the correlation nonetheless remained significant compared with the null hypothesis of no correlation [$F(1,42) = 14; 0.001 < P < 0.0005$ (Zar, 1984)].

Figure 4c, d illustrates the positive correlation between measures of time course of the action potential and absolute refractory period of the distal portions of the axon: axons with longer distal refractory periods had longer durations and longer times-to-peak. The linear correlation coefficients were 0.70 for duration versus refractory period ($n = 36$) and 0.76 for time-to-peak versus refractory period ($n = 34$), and were highly significant [$P < 0.001$, two-tailed $t$ test (Hays, 1969; Zar, 1984)] compared with the null hypothesis of no correlation.

**Intercorrelation between variables**

Because of the strong correlation existing between the two variables associated with the time course (time-to-peak and duration), it was to be expected that if one value varied with a third factor, so would the other. Similarly, given the correlation of both time course and distal refractory period with distal conduction velocity, the correlation of time course with refractory period was likely.

To determine whether there was any degree of correlation between pairs of variables that was independent of a known third factor, multiple linear correlation techniques were applied to the sub-sample of units for which all measurements were made. In this sub-sample, the data range was more restricted (conduction velocities from 15 to 49 m/s) and the conduction velocity versus absolute refractory period data could be treated assuming an underlying linear relationship ($r^2$ nearly identical for linear and exponential correlation). The time-to-peak versus conduction velocity data remained better described by a non-linear relationship, precluding analyses involving this relationship. The analysis was therefore restricted to the variables conduction velocity, absolute refractory period and duration ($n = 34$).

The first column of Table 2 lists the simple linear correlation coefficients between pairs of variables: these are nearly identical to the values obtained for the parent samples. The second column lists the coefficients of partial correlation between pairs of variables when co-variation related to the third variable (indicated in parentheses) is removed. For example, the partial correlation between conduction velocity and duration, with the influence of absolute refractory period held constant, was $-0.52$. In each of the cases, there remains a weaker, but statistically significant, correlation (compared with the null hypothesis of no correlation; see Table 2). The much smaller sizes of the partial

<table>
<thead>
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<th>Table 2. Coefficients of Correlation</th>
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<td>Simple</td>
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<tr>
<td>$r_{CV\text{-}duration}$</td>
</tr>
<tr>
<td>$r_{CV\text{-}ARP}$</td>
</tr>
<tr>
<td>$r_{ARP\text{-}duration}$</td>
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$^{*}0.02 < P < 0.05$, two-tailed, Fisher $r$ to $Z$ (Hays, 1969; Zar, 1984); $^{**}0.001 < P < 0.002$, two-tailed, Fisher $r$ to $Z$; $^{***}P < 0.001$, two-tailed, $t$ test; $CV =$ conduction velocity; $ARP =$ absolute refractory period. The values held constant in the partial correlations are listed in parentheses.
correlation coefficients compared with the simple correlation coefficients of the first column are evidence of a great degree of intercorrelation between the variables.

The effect of intercorrelation is particularly evident when the question is asked: how much more of the variability in one variable can be accounted for by knowing the values of two, rather than just one, additional variable? This is equivalent to comparing the values $R$ and $R^2$ for the multiple correlation between all three variables to the corresponding values of $|r|$ and $r^2$ for the strongest simple correlation between pairs of variables. From Table 2 it is apparent that the highest $|r|$ and $r^2$ values were obtained for the duration versus conduction velocity data: $|r| = 0.77$ and $r^2 = 0.52$. For the multiple correlation, $R$ was calculated (Hays, 1969) to be 0.81 and $R^2$ was 0.65. The values are very similar: including the additional variable led to a very modest, but significant [$F(1,31) = 5.047; P < 0.05$, $F$ test of significance of increment (Hays, 1969)] improvement in the proportion of variance accounted for. The small size of the improvement follows from the extensive intercorrelation between variables: for the purposes of accounting for or predicting one variable (e.g. duration), the remaining two variables (conduction velocity, absolute refractory period) convey much the same information.

**Correction for temperature variation**

Action potential duration (Inman and Peruzzi, 1961; Paintal, 1966), rise time (Hodgkin and Katz, 1949b; Inman and Peruzzi, 1961; Paintal, 1966) and refractory period (Paintal, 1965) have been shown to vary inversely with temperature, decreasing approximately exponentially in a temperature range including that around 27—37°C. Conduction velocity varies directly, nearly linearly, in the same range (Paintal, 1965). To test for the effects of inter- and intra-subject variations in temperature in the present data, the data were standardized to a near nerve temperature of 32.5°C, after adjusting for local variation in temperature within the hand (see Methods). Duration, time-to-peak, and absolute refractory period were corrected using experimentally determined mean $Q_{10}$ values of 3.5, 2.5 and 3.2, respectively (Paintal, 1965, 1966), in the formula $Q_{10} = \text{antilog} (10 \Delta \log X/\Delta T)$, where $X$ is the value of the parameter and $T$ is temperature (°C) (Inman and Peruzzi, 1961). For conduction velocity, the experimentally determined linear $Q_{10}$ value of 1.6 (Paintal, 1965) was used, which translates into a decrease in conduction velocity of ~4% of the value at 37°C per degree <37°C. These values for temperature-dependence of conduction velocity of single fibres are consistent with values reported for whole nerves in humans [$Q_{10}$ of 1.5 (Buchthal and Rosenfalck, 1966); reduction of 5% per degree (Johnson and Olsen, 1960)].

The results are summarized in Table 3. Mean values of conduction velocity, absolute refractory period, duration and time-to-peak calculated without correction for temperature variation are essentially identical to the values calculated after correction. Additionally, the standard deviations and ranges are nearly identical with and without temperature correction, indicating that correcting for temperature does not reduce the variability in the present data. This is also apparent from the values of the correlation coefficients: for the correlations tested, values with and without temperature correction are nearly identical. In particular, the values did not improve after correction, as would be expected if temperature variation were contributing significantly to scatter of the data. In summary, correcting for temperature did not affect the results: the extent of temperature variation
### Table 3. Comparison of Results with and Without Correction for Temperature Variation ($n = 23$)

<table>
<thead>
<tr>
<th></th>
<th>Not Corrected</th>
<th>Corrected to 32.5°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV ($\text{m/s}$)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35±11</td>
<td>35±11</td>
</tr>
<tr>
<td></td>
<td>(15–60)</td>
<td>(14–58)</td>
</tr>
<tr>
<td></td>
<td>ARP (ms)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.9±1.0</td>
<td>1.8±1.1</td>
</tr>
<tr>
<td></td>
<td>(0.7–4.5)</td>
<td>(0.8–4.7)</td>
</tr>
<tr>
<td></td>
<td>Ttp (ms)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.24±0.09</td>
<td>0.24±0.11</td>
</tr>
<tr>
<td></td>
<td>(0.12–0.48)</td>
<td>(0.13–0.52)</td>
</tr>
<tr>
<td></td>
<td>D (ms)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.49±0.11</td>
<td>0.48±0.13</td>
</tr>
<tr>
<td></td>
<td>(0.31–0.75)</td>
<td>(0.32–0.83)</td>
</tr>
</tbody>
</table>

$X \pm SD = \text{mean} \pm \text{SD}; r = \text{correlation coefficient}; *n = 27$ for CV; **$r$ for exponential relation: $-0.76$, not corrected, $-0.71$, corrected; CV = conduction velocity; ARP = absolute refractory period; D = duration; Ttp = time-to-peak.

in the present work was apparently too limited (all measurements within $5^\circ\text{C}$ of each other) for an influence of temperature to become manifest. Possibly, use of experimental average $Q_{10}$ values, rather than determining values for each fibre (impractical during the course of these studies), may have masked any temperature-dependence within this small range.

### DISCUSSION

This study presents measures of the time course of individual action potentials in man, and demonstrates that these measures (duration, time-to-peak of the positive phase) are inversely correlated to distal conduction velocity, and positively correlated to distal absolute refractory period, of the axon. The afferents studied were low-threshold mechanoreceptive afferents innervating the glabrous skin of the hand. In view of experimental animal work, it is likely that all such afferents are $A\alpha$ (also called $A\beta$) fibres (see Vallbo and Johansson, 1984), although the possibility of smaller-calibre low-threshold mechanoreceptive afferents in humans has not been excluded.

At the wrist level, where the recordings were made, axons are full calibre (Buchthal and Rosenfalck, 1966), i.e. $\sim 7–14 \ \mu\text{m}$ diameter in humans for $A\alpha$ fibres (Buchthal and Rosenfalck, 1966; Johansson and Vallbo, 1983). Converted to conduction velocities (Hursh, 1939), this corresponds to a range of $42–84 \ \text{m/s}$ at $37.5^\circ\text{C}$, or $34–67 \ \text{m/s}$ when adjusted (by the method described in Results) to the average temperature ($32.5^\circ\text{C}$) of the present data. Conduction velocities measured for action potentials evoked from the digits and recorded at the axilla are within these ranges (Johansson and Vallbo, 1983; R. G. Mackel, unpublished data), compatible with $A\alpha$ fibres. However, conduction velocities calculated from measurements made at the wrist had a lower range: from $60 \ \text{m/s}$ down to $15 \ \text{m/s}$ (at $32.5^\circ\text{C}$, present data and Mackel, 1988). Lower values for conduction velocities measured at distal, compared with proximal, sites are typically found for whole nerve responses as well (Buchthal and Rosenfalck, 1966; Kimura, 1983).
and, as discussed previously (Mackel, 1988), result from the greater weight of distal effects on the distal measure. These effects include slowing due to cooling in the fingers and slowing associated with changes in the axon (e.g. thinning, decreased internodal length) as they branch and terminate (see below and discussion in Mackel, 1988). It should also be pointed out that underestimating conduction distance would result in lower conduction velocities, with greater error for shorter distance measures.

As evident in the above discussion, conduction velocity can be influenced by local variations (e.g. environment, axon geometry) along the conduction path. However, because conduction velocity is an average measurement, the effect of local variations is diminished. For measurements made at the wrist, the values obtained are not necessarily biased toward either conduction in stem axons or conduction in more distal parts of the axons. This is because the conduction distances are short enough that distal slowing can make a significant contribution to overall conduction time, despite the fact that the afferents retain full calibre for most of the conduction distance [proximally from at least the level of the proximal phalanx for low-threshold mechanoreceptive afferents (Darian-Smith and Kenins, 1980)]. The measurement should therefore be sensitive enough to signal changes in conduction velocity that occur only in the most distal portions of the conduction path, near the end organs, as well as in the more proximal portions or throughout the conduction path.

Like conduction velocity, the absolute refractory period was measured by stimulating within the receptive field of an afferent, near its end-organs (most sensitive spot). Unlike conduction velocity, the absolute refractory period is not an average value. Absolute refractory period as defined here is the earliest time at which a second stimulus can generate and propagate a second action potential, which is detected by recording at another site along the axon. Since the action potentials must travel to be detected, any local variation along the conduction path that would cause failure of the second action potential to be propagated would affect the absolute time of the refractory period. The measurement is then of the most refractory spot along the conduction path. In the case of a uniform axon, it may be assumed that the absolute refractory period measured in this way is the absolute refractory period of the uniform axon along its length. This is not so, however, when the axon is non-uniform: for example, when stimuli are applied to terminal or near-terminal branches. Generalizing from study of normal axons in experimental animals to normal human afferents, it is likely that the axons are more refractory where they are thinner, i.e. in branches [generalizing from Paintal's observation on absolute refractory period in differently sized axons (Paintal, 1965, 1967, 1978)], or at points of step increase in axon diameter, as at branch points (Parnas et al., 1976). As mentioned above, human afferents such as those studied here remain full calibre stem axons at least into the proximal parts of the digits: branching occurs distally. Therefore, the absolute refractory periods measured here are considered to be measures of refractoriness of such distal portions of the axons. It is not assumed that these measures are indicative of the axon's refractoriness at a more proximal site (i.e. the recording site) where the axons are full calibre.

The exact sites along the axons for which absolute refractory periods have been measured are unknown, in part because the exact sites of stimulation are unknown: these can be expected to depend on innervation patterns of the afferents as well as stimulus constraints. Although not invariant, the procedure of intradermal stimulation is biased
toward activation of superficially located axonal segments, since deep insertion of the electrode, or strong stimulation, is painful (Mackel, 1988). The limited success in activating SAII and PC afferents, whose end organs are deeply located in the dermis and subcutaneously (Miller et al., 1958) suggests that, for the most part, the effects of intra-dermal stimulation are restricted to more dorsal layers. Likely targets therefore include the near-terminal and terminal branches of RA and SAI afferents innervating receptors at the undersurface of the epidermis (Cauna, 1956; Miller et al., 1958, Quilliam and Ridley, 1971), as well as larger branches and parent axons that divide within the dermis en route to the receptors (Cauna, 1956; Miller et al., 1958; Quilliam and Ridley, 1971).

In other words, a wide range of axon calibres may have been involved, which would be compatible with the wide range of absolute refractory periods that were measured. Indeed, after correction to a temperature of 37°C by the method described in the Results, the range of absolute refractory periods obtained for the present data (0.45—2.8 ms) corresponds to the range (0.45—3.2 ms) measured experimentally for the full spectrum of A fibres [(down to 1 μm (Paintal, 1978)]. In particular, the longest values are similar to absolute refractory periods of small calibre fibres, as if branching had occurred. On the other extreme, the very short refractory periods are compatible with stimulation of stem axons. Variation in axon calibre at the point of stimulation will also affect conduction velocity measurements, although the variability of these measures will be mitigated by their being average values. It is noteworthy that, despite differences in innervation patterns between RA and SAI afferents (Cauna, 1956; Miller et al., 1958; Quilliam and Ridley, 1971) that might have led to differences in distributions of axon calibres stimulated, there were no significant differences in either refractory period or conduction velocity between the two types of afferents.

In contrast to the branching shown by RA and SAI afferents, PC and SAD afferents do not branch (Quilliam and Sato, 1955; Chambers et al., 1972) or do so [SAII (Goglia and Sklenska, 1969)] in a limited fashion, so that distal stimulation of the afferents, if achieved, will most likely involve a more homogeneous group of stem axons and large calibre branches and, presumably, less variability in measurements.

The present data on distal refractory periods and distal conduction velocities, including the degree of correlation between them, are consistent with previous data (Mackel, 1988). The inverse correlation between refractory period and conduction velocity is significant and sizeable, but accounts for only half the data variability. Since the level of correlation did not improve with correction of the data for temperature variation, remaining variability could not be attributed to differential temperature sensitivities of these two parameters (see Results). One likely source of variability lies in the different nature of the two: one is a measurement on a single locus (i.e. most refractory spot); the other an average only partially sensitive to the value at that locus. However, there are no data available to verify this possibility: in particular, there are no data indicating whether or not refractory periods and conduction velocities measured where axon calibre is stable (e.g. stem axons) have a higher level of correlation. In fact, in experimental observations, measurements made at body temperature appear to be highly variable: Paintal (1965) suggested that variability may mask the correlation for fibres conducting at >25—30 m/s.

The relationship between (stem-axon) conduction velocity and absolute refractory period observed by Paintal (1965) over a wide range of A fibres (conducting at 10—80 m/s)
is non-linear, with a steep change in refractory period for slowly conducting fibres (i.e. <20 m/s) and little change for more rapidly conducting fibres. Within the range of data collected in the present study, the relationship of distal refractory period to distal conduction velocity is nearly linear: non-linear (exponential) correlation led to very modest improvement in accounting for variability. However, it is likely that the non-linear relationship would be more evident with sampling extended beyond the category of low threshold mechanoreceptive afferents to include fibres of different functions and smaller (or larger) stem-axon calibres.

Like measurements of refractory period, measurements of the time course of the action potential were made for one site along the axon: in this case, at the wrist, where the axons have full calibre. The actual quantities measured were tailored to the form of the action potential recorded by microneurography: typically a positive potential followed by a smaller, longer-lasting negative potential. The values can be expected to differ, therefore, from measurements obtained from strictly monophasic spikes recorded experimentally from cut nerves (Paintal, 1966). Thus, durations of the positive peaks of action potentials recorded from intact human afferents were slightly shorter (average 0.5 ms) than spike durations (mostly 0.6–0.7 ms) recorded from cat A α fibres (conducting faster than 30–40 m/s) at comparable temperatures [32.5°C average for present data versus 32.9°C experimental data (Paintal, 1966)]. Times-to-peak of the positive peaks were roughly comparable to the experimental rise time values [mainly 0.1–0.2 ms at 32.9°C (Paintal, 1966)].

Like the correlation of refractory period with conduction velocity, the inverse correlations of time course measures with distal conduction velocity were significant and sizeable but unable to account for roughly half the data variability. The discussion on sources of variability with respect to distal conduction velocity and refractory period measures applies also to time course measures. In experimental observations, measurements of time course made at body temperature, like those of refractory period (Paintal, 1965), show considerable variability (Paintal, 1966). This may have obscured the relationship between time course and conduction velocity in early observations (Gasser and Grundfest, 1939).

Within the range of data collected, the relationship, positive peak duration to distal conduction velocity was linear, and that of time-to-peak to distal conduction velocity slightly better described by a non-linear (power) relationship. In experimental observations collected over a wide range of conduction velocity values, the relationships of (stem-axon) time course to conduction velocity are non-linear (Paintal, 1966). Theoretical calculations suggest conduction velocity should be related to the square of the maximal rate of rise of the action potential (Hodgkin and Katz, 1949a; Hardy, 1973b), i.e. approximately proportional to the reciprocal of the square root of the time taken for the action potential to rise (Jack, 1975). In data collected from myelinated mammalian fibres, conduction velocity appears to be related to the reciprocal of time for the action potential to rise (Jack, 1975). The relationship suggested in the present data lies between the two (distal conduction velocity proportional to time-to-peak raised to −0.67). Because sizeable variability remains and because the measures of both time-to-peak and distal conduction velocity differ from the experimental measures, this observation is simply taken to indicate that the present results are roughly in line with previous experimental and theoretical results.
Action potential time course, conduction velocity and refractory period of the axon measured at the same site are related through, among other factors, common underlying ionic events. In myelinated mammalian fibres, the rapid depolarization phase of the action potential is due to a transient sodium current and repolarization due mainly to a leakage current (Chiu et al., 1979; Brismar, 1980). The time for the axon to recover its excitability after an action potential will depend on the time courses of the ionic conductance changes (Hodgkin and Huxley, 1952); that absolute refractory period lasts at least as long as the spike has long been observed (Adrian, 1921; Paintal, 1966). As mentioned above, rapidity of spread of the potential change, and therefore conduction velocity depends, in part, on rate of rise of the action potential: both are sodium sensitive (Hodgkin and Katz, 1949a; Hardy, 1973a, b). Systematic variation of membrane properties regulating the ionic currents would be expected to produce co-variation (and intercorrelation) of the measured properties. While there are many possible and probable sources of co-variation not discussed here, and while the occurrence of intercorrelation does not necessarily imply a single underlying factor, it is nonetheless possible that correlation of properties measured at the same axonal sites stems in part from common dependence on such a factor as possible size-variant density of sodium channels (e.g. discussion in Jack, 1975; and see Chiu et al., 1979).

Speculation about sources of co-variation is more difficult when the properties are measured for different sites along the axon, where axon calibre is likely to differ. The degree of correlation found may reflect the degree to which axon diameter remains stable at the measurement sites. The correlation may also depend on factors intrinsic to the particular axon and independent of dimension.

In nerve disease, axons undergo metabolic and structural changes (see Dyck et al., 1984). Changes in membrane properties affecting ionic currents (Brismar, 1983), changes in axonal geometry leading to deviation from dimensional scaling (Brill et al., 1977) and changes in local microenvironment of the axon (Brismar, 1983; Low, 1984) can affect action potential electrogenesis and propagation. Depending on the nature of the axonal change, axonal conduction properties and action potential time course might be affected in parallel, to different degrees, or diversely. Altered conduction velocity and refractoriness could affect encoding of sensory messages (Mackel, 1989). In succeeding work on neuropathy, conduction properties and action potential time course are being examined together with the responses of single afferents to natural stimulation, in an attempt to understand sensory abnormalities of neuropathy and the mechanisms that underlie them.

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