The objective of this study was to investigate the intra-examiner reliability of consecutive sensory nerve action potential measurements with short time intervals.

Amplitudes were compared in repeated sensory nerve action potential recordings of the lateral antebrachial cutaneous nerve on 63 healthy subjects. There were two sets of each two consecutive measurements, each set separated by a controlled interval of 90 minutes.

Intraclass correlation coefficients were 0.996 and 0.998 for consecutive measurements, and 0.919 and 0.926 for measurements before and after a controlled interval of 90 minutes.

The presented research clarifies the difference between measurement variability versus intra-subject variability and the influence on test-retest results in sensory nerve conduction measurements. This is of importance for correct interpretation of results when performing serial testing. It is demonstrated that a single tester can obtain reliable amplitude measurements of sensory nerve action potentials of the lateral antebrachial cutaneous nerve in asymptomatic subjects with surface recording electrodes. It is also proven that it is sufficient to control ambient temperature instead of limb temperature for this type of study on asymptomatic subjects.

**Key words**: Lateral antebrachial cutaneous nerve ; nerve conduction ; normal values ; reliability ; sensory nerve action potential.

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**Introduction**

Many clinicians monitor the amplitude of sensory nerve action potentials (SNAP) as one of the parameters for evaluating the development of the patient’s pathology. Consequently it is imperative to know the fluctuations of amplitudes in normal subjects if to make valid conclusions in serial electrophysiological testing (SET) of patients (Oh 1993). Thus far, only large time intervals between test and retest were used according to the reported studies on variability in nerve conduction measurements in SET (Bleasel, Tuck 1991 ; Bolton et al. 1981 ; Bril et al. 2001 ; Buchtal, Rosenfalck 1966 ; Chaudry et al. 1991 ; Chaudry et al. 1994 ; Salerno et al. 1995 ; Salerno et al. 1999). Buchthal et al. (1966) reported coefficients of variation of 20-30% for amplitude measurements of sensory nerve action potentials (SNAPs) in SET using needle recording electrodes. Bolton et al. (1981) reported 15% and Bleasel and Tuck (1991) 26.9% (median nerve), 32.1% (ulnar nerve) and 31.2% (sural nerve), both using surface recording electrodes. Chaudhry et al. (1991 ; 1994 ; 1995) found median and sural SNAP amplitude measurements in reference subjects and diabetic polyneuropathy patients highly reliable and internally comparable over time. They used surface recording electrodes and between test and retest was at least an interval of 1 week. Bril et al. (2001) recorded with surface electrodes the amplitude of sural SNAPs in 52 reference subjects (tested twice) and 73 diabetic polyneuropathy patients (tested 3 times). They found variability in repeated amplitude measurements of 8% in reference subjects and 10% in patients with diabetic polyneuropathy. Salerno et al. (1999) assessed intra- and inter-tester reliability of median and ulnar SNAP amplitude measurements in active workers. Two examiners were involved and re-assessment was done after 3 weeks. Intra-observer reliability had intraclass correlation coefficients (ICC) of 0.88 and 0.81 (respectively for tester 1 and 2) for median SNAP amplitudes, and 0.68 and 0.80 (respectively for tester 1 and 2) for ulnar SNAP amplitudes.

In spite of these reports it is still not clear to which extent factors contribute to the mentioned results since the fluctuations in amplitudes of SNAPs depend on the variability of the subject and on measurement errors (Oh 1993). The latter consists of a combination of various technical and physiological factors and they can only be distinguished from the variability of the subject by using short time intervals between test and retest. This research was conducted to clarify this issue by

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using consecutive amplitude measurements of SNAPs of the lateral antebrachial cutaneous nerve using surface recording electrodes. Two situations were explored: one with immediate test-retest in order to study measurement variability and one with a controlled interval of 90 minutes to study the influence of measurement variability in combination with intra-subject variability. The hypothesis was that better results can be obtained than those reported in literature by reducing the intra-subject-variability and by using a stringent protocol to minimise measurement error.

Also the use of ambient temperature control versus limb temperature control was challenged hypothesising that room temperature control is adequate for this type of study on asymptomatic subjects.

**Methods**

**Subjects**

One examiner tested 63 healthy volunteers (11 men, 52 women, age between 19-25 years, BMI between 16.11-24.83 kg/m²) after obtaining written informed consent. Exclusion criteria such as diabetes, neuropathy, radicular syndrome, peripheral nerve damage, sensory disturbance in upper extremities and peripheral oedema were explored by means of a questionnaire.

**Materials**

The nerve conduction recordings were made with a Medelec Neurostar MS 92 B (Oxford Instruments, Old Woking, United Kingdom) using 20 Hz-2 kHz filter settings, 0.1-ms square-wave pulses, 20-ms sweep duration and a repetition rate of 1 Hz.

The nerves were stimulated with a Medelec bipolar nerve stimulation electrode from Oxford Instruments (Old Woking, United Kingdom) with 6 mm diameter felt pads soaked in physiological saline and an inter-electrode separation of 25 mm.

The recording and ground electrodes were specially made with silver strips (95% Ag, 5% Cu), coated with Dracard conductive electrode gel. The recording electrodes were 5 by 5 mm, and 0.2 mm thick; they were mounted together on a plastic bar with an inter-electrode separation of 30 mm. The ground electrode was 30 by 5 mm, 0.2 mm thick, and mounted on a separate plastic bar.

Ambient temperature was monitored with a Comark (Welwyn Garden City, United Kingdom) C9001 thermometer with AT27M type T thermocouple. Skin impedance was measured with an E0001 electrode impedance meter (37-Hz nominal) from SLE (South Croydon, United Kingdom).

**Procedure**

A preliminary study on the influence of room temperature on skin temperature was performed to verify our hypothesis that is not required to control skin temperature in this type of study on healthy subjects if the ambient temperature is kept stable. After an adaptation period of 15 minutes to the room temperature, skin temperature was measured every 10 minutes at the posterior side of the distal forearm of 10 subjects during 90 minutes. With an ambient temperature between 17.4 °C and 18.7 °C, skin temperature fluctuated between 0.5 °C (subject with least fluctuations: between 24.9 °C and 25.4 °C) and 1.8 °C (subject with most fluctuations: between 29.7 °C and 31.5 °C).

After measuring physical height and body weight, the subject was seated with the supported left forearm in supination.

A constant ambient temperature, monitored 1 m beside the subject and kept below 20 °C (mean, 18.2 °C) during the whole procedure, combined with light clothing, minimised skin sympathetic reflex activity and resulting SNAP amplitude fluctuations (Sawasaki et al. 2001; Iwase et al. 2002). Pressure on the recording electrodes was kept the same, since this is also an influencing factor (Ven et al. 2004).

Some of the features in this study like the choice of the nerve (the lateral antebrachial cutaneous nerve), the othodromic way of stimulation, the use of handheld recording electrodes and the measurement of only amplitudes and no latencies were specifically chosen because this research fits into a series of studies we performed investigating the influence of physiological and non-physiological factors on amplitude measurements of sensory nerve action potentials (Ven et al. 2004).

The skin of the ventral side of the left forearm was thoroughly scrubbed with a pumice paste and cleaned with ethanol until the recorded impedance was below 20 kW.

Stimulating electrodes were attached by a Velcro® strap to the wrist radial to the flexor carpi radialis tendon; the cathode was 140 mm distal to the active recording electrode and the anode more distal. The ground electrode was taped on the skin midway between the location of the recording and stimulating electrodes. The recording electrodes were handled for maximal amplitude detection and a measurement was recorded on the location where the highest SNAP amplitude could be obtained; the active electrode 40 mm distal to the elbow fold and the reference electrode more proximal. Stimuli were supramaximal and averaging technique was used to amplify the signal to noise ratio. SNAP amplitudes were measured between the negative peak and the preceding positive peak.

A SNAP (1a) was measured. All electrodes were removed; skin and electrodes cleaned. Conductive gel was reapplied and all electrodes were immediately repositioned. Again a SNAP (1b) was measured. Following an interval of 90 minutes during which all subjects were submitted to the same passive protocol, all electrodes were repositioned...
and a SNAP (2a) was measured. All electrodes were removed; skin and electrodes cleaned. Conductive gel was reapplied and all electrodes were immediately repositioned. Again a SNAP (2b) was measured.

**Statistical Analysis**

Bland-Altman plots were constructed to visualise differences between two amplitude measurements against the mean of the measurements, situating the obtained data and the accepted limits of association for the population. Intraclass correlation coefficients with a standard error (SE) and mean SNAP values with a standard deviation (SD) were calculated. All analyses were performed with the SAS-statistical package (version 8.1) (SAS Institute Inc., Cary, North Carolina).

**Results**

Visualised in the Bland-Altman plots, mean difference between test-retest equals 0.0 mV (1a, 1b; Fig. 1a; and 2a, 2b; Fig. 1b) and 95% of the test-retest differences are located between -1.5 mV and 1.4 mV (1a, 1b) and between -0.9 mV and 0.9 mV (2a, 2b).

ICC between test-retest without interval is 0.996 with a SE of 0.001 (1a, 1b) and 0.998 with a SE of 0.000 (2a, 2b). ICC between test-retest with an interval of 90 minutes is 0.926 with a SE of 0.018 (1a, 2a) and 0.919 with a SE of 0.019 (1b, 2b).

Mean SNAP values are 20.2 mV with a SD of 7.9 (1a), 20.2 mV with a SD of 7.9 (1b), 21.1 mV with a SD of 8.1 (2a), and 21.0 mV with a SD of 8.2 (2b).

**Discussion**

In our study two times two consecutive measurements without time interval, and a test-retest with a controlled time interval of 90 minutes, were performed with excellent (Fleiss 1981) correlations. With the controlled 90 minutes time interval between test and retest, correlations were slightly lower than in the previous condition, primarily due to the influence of intra-subject variability. These findings allow for a correct interpretation of the research results described in the literature (Bleasel, Tuck 1991; Bolton et al. 1981; Bril et al. 2001; Buchtal, Rosenfalck 1966; Chaudry et al. 1991; Chaudry et al. 1994; Chaudry et al. 1995; Salerno et al. 1999), where the lower correlations have to be the consequence of predominantly intra-subject variability.

In the reviewed literature some authors (Bleasel, Tuck 1991; Chaudry et al. 1991; Chaudry et al. 1994; Chaudry et al. 1995) used the term intra-examiner reliability, others (Bolton et al. 1981; Buchtal, Rosenfalck 1966) the term intra-subject variability in test-retest reliability studies of SNAP amplitude measurements.

According to statistical literature (Dawson-Saunders, Trapp 1994; Everitt 1994; Hopkins 2000), reliability is the extent of agreement between repeated measurements (repeatability) on the same subject, which depends on the true score (variability of the measured characteristic) and the error variability. The latter consists on the one hand of variability introduced by the instrument (or method) and the examiner (in this study designated as measurement variability), and on the other hand of variability due to environmental and biological changes of the subject (in this study designated as intra-subject variability).

To perform and report reliability studies, an appropriate research design and correct terminology seems important. Minimal time interval should be used to study measurement variability, larger time intervals between test and retest (as in SET) to
investigate intra-subject variability, provided measurement variability is proved to be very low (as was shown in our study).

In many clinics and research centres, it is a standard to measure and sometimes adapt skin temperature or correct for it using conversion tables. However, according to the studies of Sawasaki et al. (2001) and Iwase et al. (2002) local warming or cooling from the skin does not exclude the influence of central mechanisms which can induce skin sympathetic reflex activity. Since these mechanisms can have an effect on SNAP amplitudes and as skin temperature control is not so easy because local warming-up and cooling-down takes time before the limb temperature becomes stable, we explored the use of room temperature control for our study. The choice of a 15 minute room adaptation period before measurements and a rather cool environment to limit skin sympathetic reflex activity and therefore SNAP amplitude fluctuations were based on the results of the mentioned studies of Sawasaki et al. (2001) and Iwase et al. (2002). The excellent correlations between test and retest in our study demonstrate small variability which is only possible if the selected procedure was adequate for its purpose. Since the influence of environmental temperature on skin temperature and on SNAP amplitude fluctuations was not the subject of our study, we only want to open up the discussion whether to use local warming and cooling and/or room temperature control in nerve conduction studies. Further detailed research considering this issue like a correlation study between room temperature, skin temperature and SNAP measurements seems required. This might provide more detailed information on issues like the circumstances under which it would be preferable to use local or room temperature control (e.g. our study was performed on asymptomatic subjects and can’t be just transferred to patients with pathological limb temperature conditions like Raynaud’s disease), how long the room adaptation period should be, if there is a difference between the forearm -as in our study- and for instance fingers or toes that might have bigger skin temperature fluctuations, the optimal room temperature and the allowed room temperature fluctuations.

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