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Research paper

A test to determine the site of abnormal neuromuscular refractoriness

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ABSTRACT

Objective: The relative refractory period (RRP) of motor axons is an important parameter in nerve excitability tests of the recovery cycle (RC). Abnormalities may have a site in the axonal membrane, the neuromuscular junction, or in a dysfunction of the muscle. We aimed in this study to determine the site of abnormality, using a modified protocol of the conventional RC test, whereby an additional supramaximal stimulus is added at the same interstimulus interval as in RC recordings (RCSM).
Methods: Twenty-four healthy subjects aged 37.8 ± 2.4 years (mean ± SE) were examined with median nerve excitability testing using RC and RCSM protocols at normal temperature (34.1 ± 0.2 °C). The recordings were repeated in 12 subjects after selective cooling of the thenar muscle (25.2 ± 0.7 °C) and in 12 subjects after cooling the nerve trunk at the wrist (24.9 ± 0.3 °C).
Results: After cooling the nerve, RRP measured with RC and RCSM were prolonged similarly (medians by 1.8 ms, and 2.1 ms respectively). In contrast, cooling the muscle prolonged RRP measured with RC (by 1.3 ms), but did not significantly prolong RRP measured with RCSM. RRPs measured by RC and RCSM were significantly different when cooling was at the muscle (P = 5.10^{-4}), but not when cooling was at the nerve (P = 0.57).
Conclusions: A difference between RC and RCSM indicates abnormal excitability distal to the axonal membrane under the stimulating electrode.
Significance: Combining RCSM with the conventional RC protocol should help to localize the site of abnormal neuromuscular refractoriness.

1. Introduction

The recovery cycle (RC) of nerve excitability in motor and sensory axons, like threshold electrotonus, provides useful complementary information to nerve conduction studies (Kiernan et al., 2020). Three components can be found in the recovery cycle of excitability: refractoriness, superexcitability, and late subexcitability. This study will focus on refractoriness, which is commonly expressed in terms of the relative refractory period (RRP), the interval between a supramaximal conditioning stimulus and the test stimulus at which excitability returns to its baseline value, and refractoriness switches to superexcitability. The RRP is prolonged by membrane depolarization and ischaemia (Kiernan and Bostock, 2000) and prolongation is a common finding in nerve excitability tests of patients with axonal Guillain-Barre syndrome

(Kuwabara et al., 2003), uremic neuropathy (Krishnan et al., 2005) and after treatment with oxaliplatin (Park et al., 2011; Heide et al., 2018; Bennedsgaard et al., 2020). On the other hand it can also be decreased, as in episodic ataxia type 1 (Tomlinson et al., 2010) and multifocal motor neuropathy (Kovalchuk et al., 2020). A problem in the interpretation of an abnormal RRP is that axons, the neuromuscular junction or the muscle membrane may cause the alteration. For example, abnormal RRP has been shown in myotonic dystrophy type 1 (Boërio et al., 2007), inclusion body myositis (Lee et al., 2020) and in critically ill patients (Z'Graggen et al., 2006), but the site of alteration of RC was not determined.

The study of the RC in motor axons requires two compound muscle action potentials (CMAPs) produced by two electrical stimuli at peripheral nerves within a short time interval (2 – 200 ms). The first, supramaximal stimulus, is combined with a second stimulus set to a target amplitude at 40% of the first stimulus. Here, we describe a modified test for the recording of the RC (RCSM), in which the target response amplitude is set to 40% of the response to a supramaximal stimulus at the same interstimulus interval. This overcomes the problem with the conventional RC protocol,

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that if the muscle or neuromuscular junction become unusually refractory, then the maximum conditioned response is reduced, so that the threshold current required to generate the normal target response is increased at short interstimulus intervals, and may even become infinite.

In the present study, we aimed to test whether the additional RCSM protocol enables one to determine whether the site of the abnormal membrane excitability is in the axonal membrane under the stimulating electrode, or more distal, such as the neuromuscular junction or the muscle membrane. We applied focal cooling of the muscle and nerve separately, to evaluate the ability of the protocol to discriminate whether the pathology is in the nerve or muscle.

2. Methods

2.1. Participants

All examinations were undertaken at the Department of Clinical Neurophysiology, Aarhus University Hospital. In total, 24 healthy subjects were included. Subjects with a history of diabetes, malignancy, use of medication that effects peripheral nervous system, alcohol abuse or known polyneuropathy were excluded. Additionally, carpal tunnel syndrome was excluded with conventional nerve conduction studies and standard methods (Stålberg et al., 2019) using Keypoint EMG equipment 2.11 (Dantec, Skovlunde, Denmark).

Right side was examined in all subjects. In all 24 subjects RC and RCSM protocols were applied at normal skin temperature of between 32 and 35 °C by warming the wrist and the muscle with a heating lamp when necessary (experiment 1). Then, in 12 subjects the thenar muscle was focally cooled (experiment 2), and in the other 12 subjects the nerve trunk at the wrist was focally cooled (experiment 3). Skin temperature was measured with an infrared skin thermometer.

The participants experienced the RCSM protocol as slightly more unpleasant, but all subjects could complete all examinations.

All participants gave written informed consent in accordance with the Declaration of Helsinki II. The project was approved by The Central Denmark Region Committees on Health Research Ethics.

2.2. Nerve excitability test

The study is based on the consensus guidelines for measurements of axonal excitability (Kiernan et al., 2020). In brief, QTRACW software (© Institute of Neurology, London) was available on a laptop with a Windows operating system. The other components of the set-up were a DS5 isolated bipolar constant current stimulator (Digitimer Ltd; Welwyn Garden City, UK), National Instruments USB-6251-BNC data acquisition device (National Instruments, Hørsholm, DK), the HumBug 50 Hz noise eliminator (Digitimer Ltd.,), and a 2-channel isolated amplifier (D440-2, Digitimer Ltd.,) with a bandpass filter of 3 Hz – 3 kHz and a gain of 300. Stimulus current was applied using two non-polarizable self-adhesive electrodes (Ambu® BlueSensor QR), the cathode placed at the wrist at the site of lowest stimulation threshold and the anode is placed 10 cm proximal to the first stimulating electrode, ensuring the placement is not over the median nerve. The recordings were performed using surface electrodes (Ambu® BlueSensor NF) in the abductor pollicis brevis muscle with the active electrode over the motor point and a reference electrode

placed at the metacarpo-phalangeal joint. The distance between the recording and stimulation sites was between 60 and 70 mm. A ground electrode (Ambu® Neuroline Ground) was placed at the dorsum of the hand.

2.3. Recovery cycle

The TRONDNF nerve excitability protocol of the QTRACW software provides for both conventional measurements of the RC, in which the target response threshold remains constant, and also for the modification described here (RCSM), in which the target response threshold depends on the response to an additional supramaximal stimulus. In detail, in RC, three different channels for nerve stimulation are used (Fig. 1A-D): In one channel, the CMAP with a supramaximal stimulation is recorded (Fig. 1B). Two other test channels determine the current necessary to elicit a CMAP with a target set to 40% of the maximal amplitude (Fig. 1A, 1D). Such tests are applied either alone (test only, (Fig. 1A) or after an unconditioned supramaximal stimulus at an interstimulus interval of 2–200 ms (Fig. 1D). RCSM, on the other hand, uses an additional stimulation channel (Fig. 1C). This sets the target response amplitude to 40% of the response to an additional supramaximal stimulus at the same interstimulus interval (Fig. 1D).

2.4. Temperature control

In the first series of experiments, recordings were made at a skin temperature above 32 °C (experiment 1). Then, the experiments were repeated using focal thenar muscle cooling (experiment 2) or cooling at the wrist (experiment 3) to a skin temperature of around 25 °C. The cooling was done using Nexcare ColdHot Mini which was kept in the freezer at least for 12 h before the experiments. The cooled Nexcare ColdHot Mini is covered with a layer of cotton cloth during the experiments to avoid fast cooling at the cooling site and remote cooling at the non-cooled site. It took around 3–5 min to cool the nerve and the muscle. The Nexcare ColdHot Mini was only removed when measuring the temperature, or if the temperature was below 25 °C, or if the subject felt that it was unpleasant.

2.5. Statistics

The statistical analysis protocols in QTRACW were used for basic statistics. Unless otherwise indicated, group values are given by means ± SE. Since RRPs are not normally distributed, however, calculations of significance for differences in medians were performed by Wilcoxon signed ranks tests. Levels of significance in the figures are indicated by stars; * $p = < 0.05$, ** $p = < 0.01$, *** $p = < 0.001$; n.s. not significant.

3. Results

3.1. Cooling of muscle

In this series of experiments, the two nerve excitability tests (RC, RCSM) were applied before and after cooling of the muscle only (12 control subjects with a mean age of 37.2 ± 2.96 years). A representative example of such recordings during cooling of the muscle is given in Fig. 2. Illustrated are changes in the CMAP amplitudes obtained with the various stimulation channels (see Fig. 1) during shortening of the interstimulus interval from 200
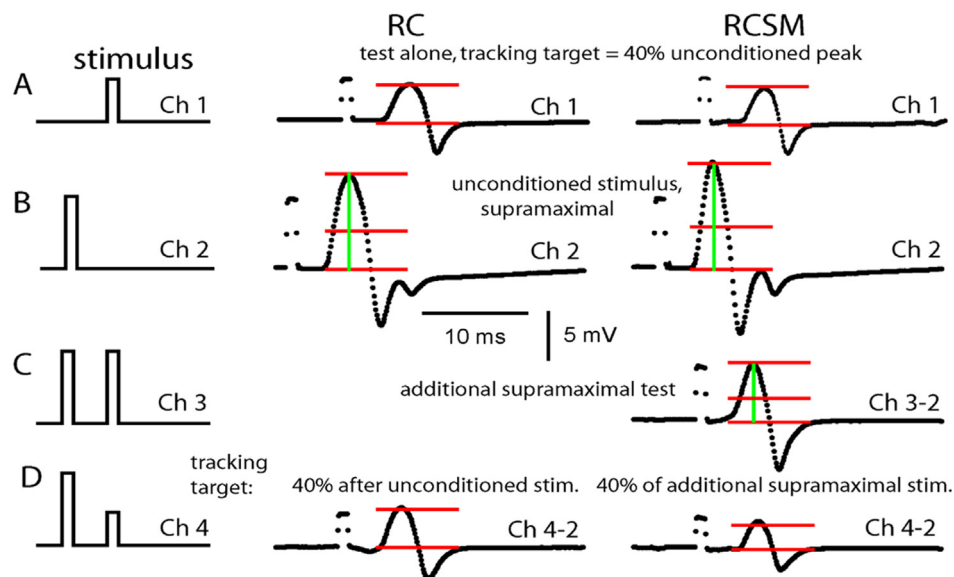
Fig. 1. Two different protocols for nerve excitability testing were used: RC for the conventional recovery cycle of excitability; RCSM with an additional supramaximal stimulus. A. Test alone, tracking target = 40% unconditioned peak. B. Conditioning stimulus alone, set to 1.7 × test stimulus in channel 1 (i.e. supramaximal). C. Conditioning + supramaximal test, both set to 1.7 × test stimulus channel 1. Peak height measured after subtracting response from channel 2 (RCSM only). D. Conditioning + test; (RC) tracking target set to 40% of peak on channel 2; (RCSM) tracking target set to 40% of peak on channel 3, peak height measured after subtracting response from channel 2. The red bars indicate the time frames in which the peak heights of the CMAPs were measured. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to 2 ms. The RCs show the alterations in the threshold currents necessary to maintain a test CMAP at 40% of an unconditioned supramaximal CMAP (RC) or at 40% of a conditioned supramaximal CMAP at the same interstimulus interval (RCSM).

The statistical analysis of averaged data obtained from 12 subjects before and after cooling is illustrated in Fig. 3. RC and RCSM were tested with mean skin temperatures at the thenar muscle of 34.6 °C or 25.2 °C. This resulted in a prolongation of the RRP from 2.95 ± 0.09 to 4.39 ± 0.29 ms when measured by RC and from 2.81 ± 0.08 to 3.11 ± 0.14 ms when measured with RCSM. The difference in medians between RRP after cooling measured by the two protocols (1.36 ms) is highly significant (P = 5.10^{-4}); see Fig. 4.

3.2. Cooling of wrist

In the second series of experiments, RC and RCSM were tested after cooling of the wrist (12 subjects with a mean age of 38.3 ± 3.8 years). The statistical analysis of averaged data obtained in such recordings is illustrated in Fig. 3. RC and RCSM were tested at mean skin temperatures at the wrist of 33.5 °C or 25.1 °C. Cooling resulted in a prolongation of the mean RRP from 3.16 ± 0.1 to 5.08 ± 0.27 ms when measured by RC and from 3.05 ± 0.09 to 5.23 ± 0.30 ms when recorded with RCSM. After cooling at the wrist, in contrast to the muscle, the difference in RRP measured by the two protocols is statistically not significant (p = 0.57) (see Fig. 4).

3.3. Group data

A summary of group data is illustrated in Fig. 5. The figure shows the complete recovery cycles of all groups tested in this study. Illustrated are data of all 24 participants tested with RC and RCSM at a warm temperature (A). In a second group, 12 of the participants were tested with both protocols before and after cooling at the muscle (B, C). The third group data were recorded

from 12 of the participants using RC and RCSM before and after cooling at the wrist (D, E).

4. Discussion:

4.1. Relative refractory period

A key component in the RC of axonal excitability is the early RRP. In the past, this parameter has been tested in several types of neuropathy (Kuwabara et al., 2003; Boerio et al., 2004, 2005; Kiernan et al., 2020). It is generally accepted that the major determinant of the recovery from refractoriness in normal mammalian myelinated axons is the recovery of Na + channels from inactivation (Kiernan et al., 2020). Conventional tests of refractoriness, however, do not allow to clearly identify the site along the motor unit responsible for such an abnormality. It could be due to a focal pathology, a general dysfunction of the axonal membrane, the neuromuscular junction, or the muscle membrane.

In the present study, we have tested the hypothesis that the RCSM protocol can differentiate between changes in the axonal membrane under the stimulating electrode and changes occurring elsewhere. For this purpose, selective cooling was applied at the muscle or at the wrist. In previous studies, changes in temperature were produced by cooling of the arm in a water bath (Kiernan et al., 2001, Franssen et al., 2010). It is a general finding that such a procedure results in a prolongation of RRP. This method of cooling, however, may have affected the recovery of Na + channels from inactivation in the axonal trunk, but also the nerve terminal and/ or the muscle membrane. In the present study, the muscle or the nerve trunk at the wrist were cooled selectively.

The comparison between RC and RCSM revealed clear differences. The RCSM protocol was much less affected by cooling of the muscle only. This effect was not seen when the nerve trunk was cooled at the wrist, i.e. at the stimulating cathode (see Fig. 3,4). This indicates that the additional supramaximal stimulus
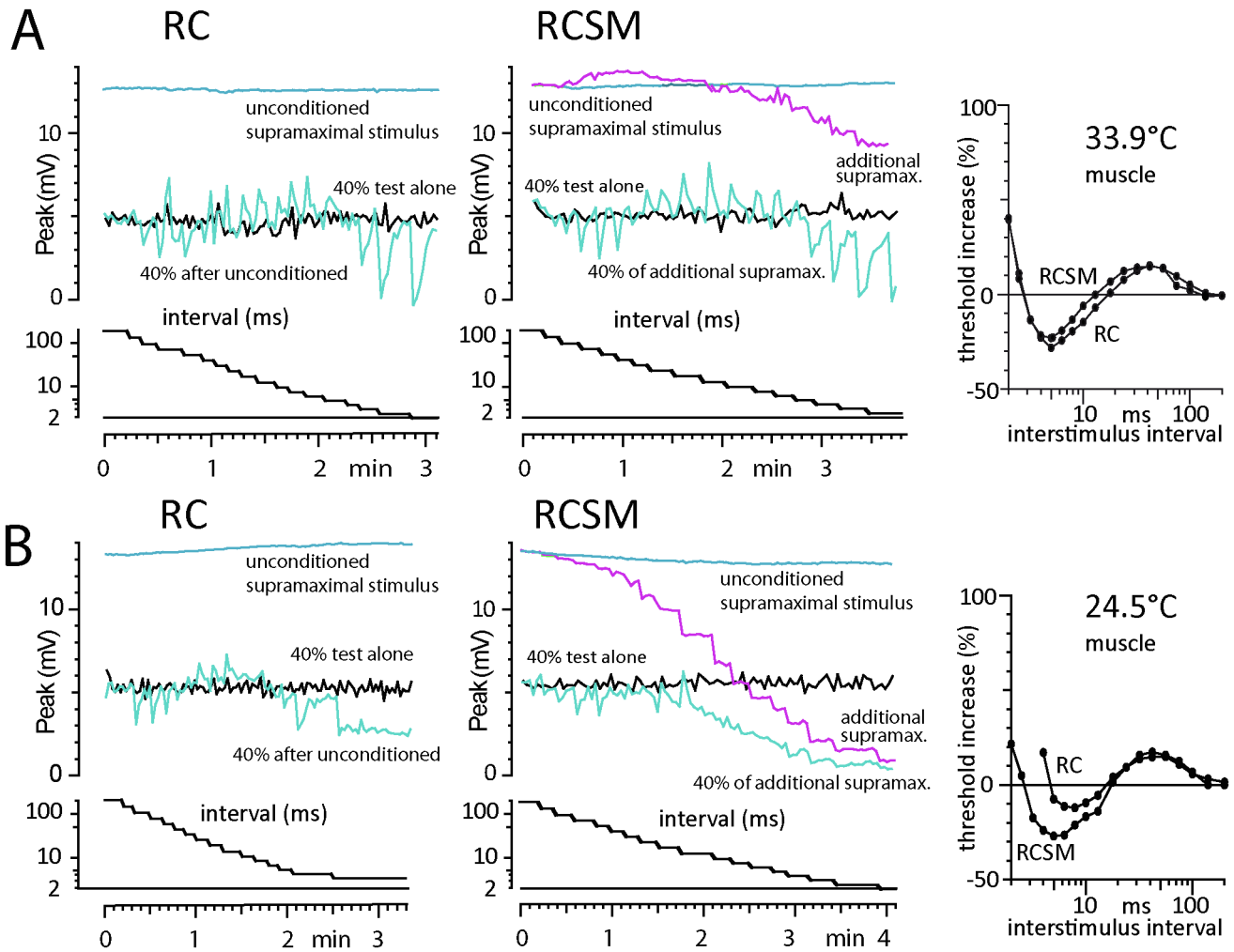
Fig. 2. A representative example of one of the participants for changes in peak amplitudes and threshold currents of the different stimulation channels in relation to the interstimulus interval and at different temperatures. The interval between the unconditioned supramaximal stimulus and the following test stimulus with 40% in amplitude are plotted with log scales in the left and center columns. A Illustrated are the peak amplitudes of the CMAPs recorded with the different stimulation channels with the RC and RCSM protocols given in Fig. 1. The recovery cycles of threshold currents for both protocols at a skin temperature of 33.9 °C at the thenar muscle are plotted in the graph to the right. B Illustrated are the identical parameters given in A at a skin temperature of 24.5 °C at the thenar muscle. Note, that at the low temperature, the 40% tracking target of the unconditioned supramaximal test stimulus in the RC recording is not reached at short interstimulus intervals.
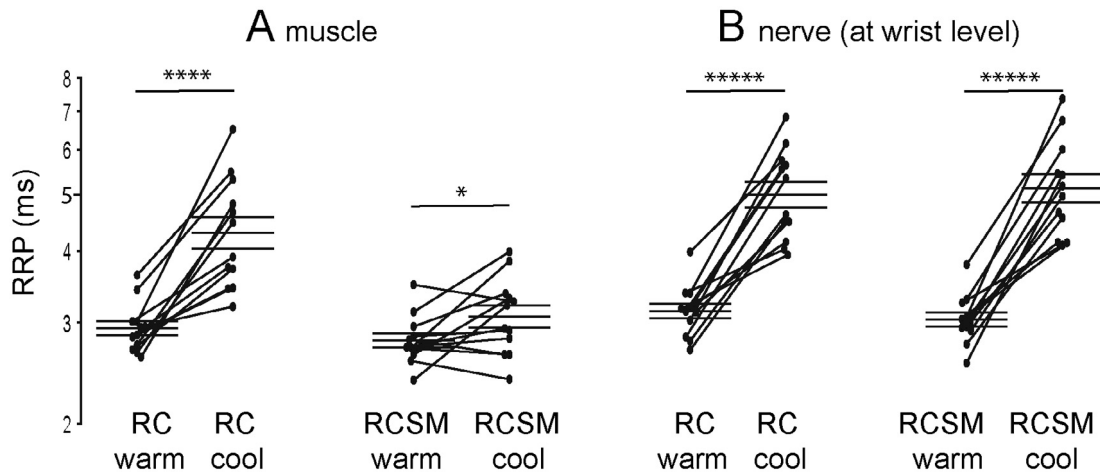

Fig. 3. Summary of changes in the relative refractory period determined with the RC and RCSM protocols. Note, Y-axis with logarithmic scale. In each case, 12 healthy participants were tested by both protocols in one recording session either before and after cooling of the thenar muscle or of the median nerve at the wrist. The horizontal bars indicate medians and interquartile ranges. The statistical significance levels are indicated by stars.
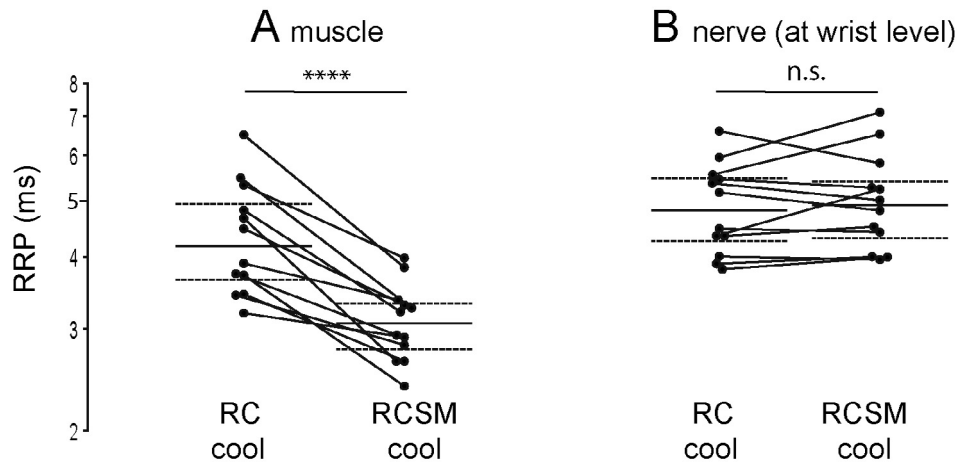
Fig. 4. The RCSM, but not the RC protocol, can disclose the site of an abnormality in the recovery cycle of excitability. Illustrated are the relative refractory periods (RRP) recorded by both protocols. Note, Y-axis with logarithmic scale. In each case, 12 healthy participants were tested by both protocols in one recording session after cooling of the thenar muscle or of the median nerve at the wrist. The horizontal bars indicate medians and interquartile ranges. The statistical significance levels are indicated by stars. n.s.: not significant. Note, that an abnormally long-lasting RRP is seen after cooling of the muscle or of the wrist when measured by the RC protocol. With the use of the RCSM protocol, however, this change in the early recovery period is only seen after cooling of the wrist, i.e. at the site of the stimulating electrode.

can compensate for most of the prolongation in RRP at a site outside the membrane under the stimulating electrode.

4.2. Differences between RC and RCSM at warm temperatures

The data indicate that a small difference in RRP measured by RC and RCSM may be already observed in normal conditions, suggesting that refractoriness distal to the site of stimulation makes a small contribution to the RRP as conventionally recorded. We observed a maximal difference of 0.3 ms in the 24 normal subjects tested. In contrast, up to 2.8 ms difference in RC and RCSM were observed after cooling of the muscle (see Fig. 3). It may be useful, therefore, to set a limit for the difference in recordings of RRP measured by RC and RCSM in patients, beyond which a distal source of refractoriness can safely be inferred.
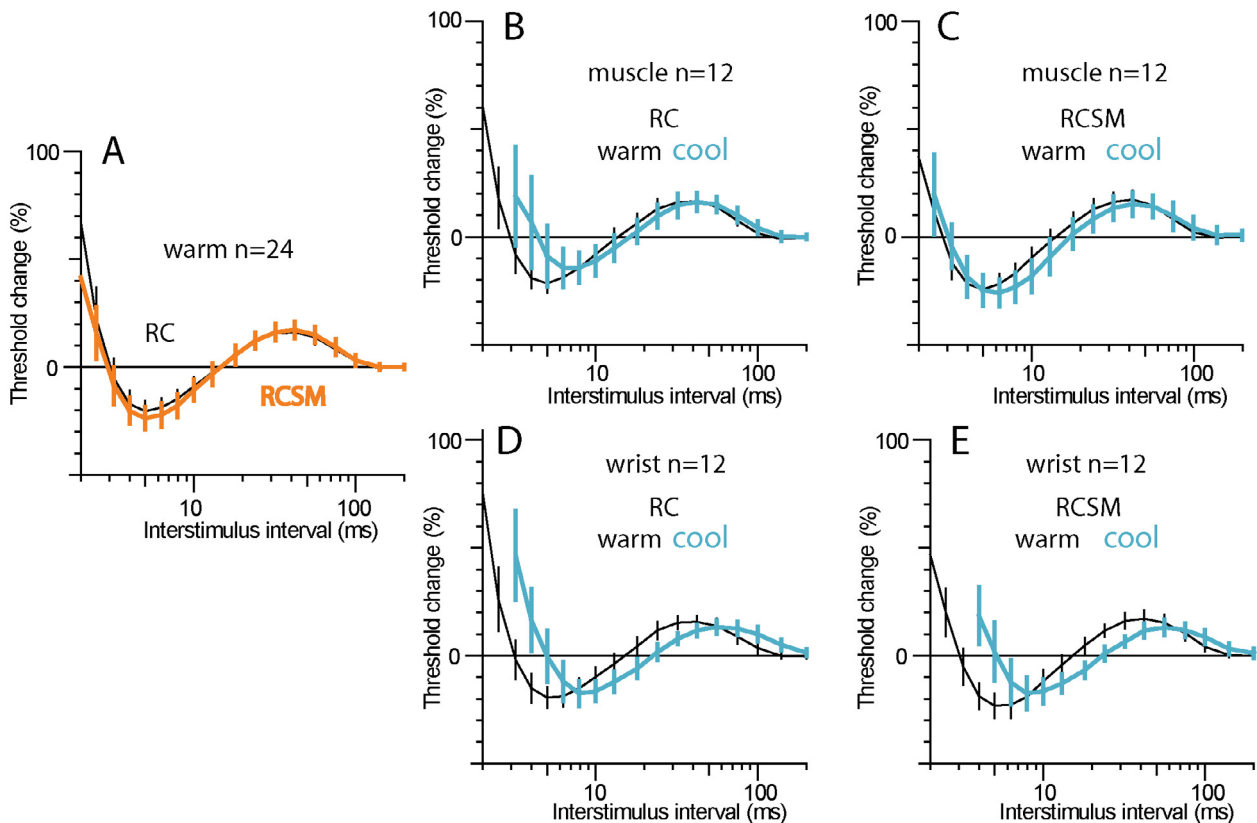

Fig. 5. Summary of group data. The figure shows the complete recovery cycles of all groups tested in this study. Illustrated are data of all 24 participants tested with RC and RCSM at a warm temperature (A). In a second group, 12 of the participants were tested with RC and RCSM before and after cooling at the muscle (B, C). The third group data were recorded from 12 of the participants using RC and RCSM before and after cooling at the wrist (D, E).

Additionally, our study shows once more the importance of optimal temperature during nerve excitability testing. Similar to conventional RC and other nerve excitability protocols, the temperature should be kept stable around 32° for RCSM protocol as well.

4.3. Implications for mathematical modelling

Mathematical modelling is sometimes used as an aid to the interpretation of nerve excitability measurements, but as was noted in the recent consensus guidelines (Kiernan et al., 2020), 'if patient excitability properties are only abnormal in refractoriness, the defect is likely to be confined to the motor nerve terminals, neuromuscular junction or muscle, and modelling nerve excitability in the nerve trunk will be unhelpful'. The RCSM protocol, that allows for the contributions of these distal parts of the motor unit to refractoriness, enables recovery cycle measurements to more accurately reflect the properties of axons at the site of stimulation, and is therefore strongly recommended if modelling is anticipated. It is arguable that the RCSM protocol should replace the conventional RC one as the first method of choice, even when distal problems are not suspected, since the additional strong stimuli are usually well tolerated, and the recovery cycle should be more accurate.

5. Limitations

Cooling of the nerve and muscle were done in two different groups of subjects, but there is no reason to suppose that the results would have been any different if the same subjects were used for both series of experiments. This study does not describe observations on patients with neuropathy, neuromuscular transmission disorders or myopathy, but it is assumed that the focal cooling used adequately simulates pathological refractoriness arising at the same sites.

6. Conclusions

In contrast to the conventional RC protocol, the RCSM protocol records nerve recovery cycles at the site stimulated, relatively undistorted by distal transmission. A marked difference in RRP between RCSM and RC therefore indicates that the abnormal refractoriness is arising distally.

Funding

Declaration of competing interest

H.Bostock receives from UCL a share of the royalties for sales of the QtracW software used in this study. Other authors have no conflicts of interest to declare.

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