Clinical Neurophysiology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Measurement of axonal excitability: Consensus guidelines

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ARTICLE INFO

Article history:
Accepted 24 July 2019
Available online xxxx

Keywords:
Excitability
Peripheral nerve
Axon
Threshold
Guidelines

HIGHLIGHTS

- Axonal excitability studies allow deductions about membrane and ion channel properties *in vivo.*
- Excitability studies have investigated nerve function and pathophysiology in neurological disease.
- Guidelines summarise physiological basis, methodology and interpretation of excitability studies.

ABSTRACT

Measurement of axonal excitability provides an *in vivo* indication of the properties of the nerve membrane and of the ion channels expressed on these axons. Axonal excitability techniques have been utilised to investigate the pathophysiological mechanisms underlying neurological diseases. This document presents guidelines derived for such studies, based on a consensus of international experts, and highlights the potential difficulties when interpreting abnormalities in diseased axons. The present manuscript provides a state-of-the-art review of the findings of axonal excitability studies and their interpretation, in addition to suggesting guidelines for the optimal performance of excitability studies.

Contents

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https://doi.org/10.1016/j.clinph.2019.07.023
1388-2457/

Please cite this article as: M. C. Kiernan, H. Bostock, S. B. Park et al., Measurement of axonal excitability: Consensus guidelines, Clinical Neurophysiology, https://doi.org/10.1016/j.clinph.2019.07.023

1. Introduction

Axonal excitability techniques provide information about ion channel function and surrogate markers of axonal membrane potential *in vivo* in human axons. As a neurophysiological technique, axonal excitability studies have become more available in clinical and research settings over the past 20 years, enabling investigation of the properties of the nerve membrane as well as pathophysiological mechanisms underlying neurological diseases. In contrast to nerve conduction studies, axonal excitability studies utilise submaximal stimuli to examine the properties underlying the excitability of the axon. While nerve conduction studies measure impulse conduction, specifically the ability of axons to conduct between the stimulating and recording sites, axonal excitability studies provide insight into the properties of the axonal membrane at the site of stimulation. Given the translation and significant uptake of axonal excitability techniques in practices worldwide, it seems timely to produce a set of guidelines based on a consensus of international experts.

Current axonal excitability protocols utilise threshold tracking as a preferred technique (reviewed in Bostock et al., 1998). The basic premise of threshold tracking techniques is to measure the strength of the stimulus required to produce a compound action potential (CAP) of a specified size, termed the "threshold". Typically the target selected corresponds to 30–40% of the maximum CAP, which sits on the steepest portion of the stimulus-response curve and is most responsive to change (Kiernan et al., 2000, 2001a). When the test response is smaller than the tracking target, the intensity of the subsequent stimulus is increased, and conversely when the response is larger than the tracking target it is reduced (Bostock et al., 1998; Burke et al., 2001). The target size may be set to any size, but the extreme ends of the stimulus-response curve should be avoided because large changes in current then produce small changes in the recorded potential. Changing the stimulus intensity so that the test potential remains the same size during different manoeuvres avoids "floor" and "ceiling" effects and furthermore, enables the study of axons of similar size despite changes in their excitability (Bostock et al., 1998; Burke et al., 2001).

Most studies using threshold tracking have been undertaken on large myelinated axons, revealing the properties of the nerve membrane and of the ion channels expressed on these axons (Fig. 1; Table 1). As with nerve conduction studies, small myelinated axons cannot be readily studied using the excitability techniques

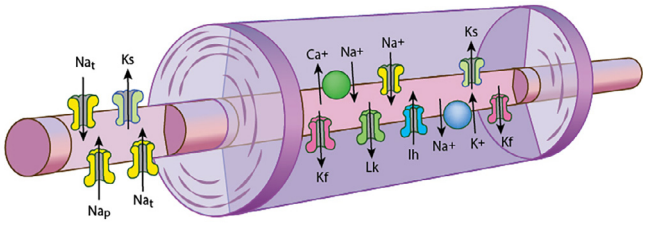

Fig. 1. Ion channel distribution and axonal structure. Schematic diagram with high density of voltage gated Na^+ channels (both transient (Na_t) and persistent (Na_p)) at the node of Ranvier. Slow K^+ channels (K_s) are also depicted at the node. Fast K^+ channels (K_f) located adjacent to the node in the internode under the myelin sheath, as well as the hyperpolarization activated cation conductance (I_h), slow K^+ channels and voltage-independent leak conductances (Lk), Na^+/K^+ pump and the Na^+/Ca^{2+} exchanger.

Table 1
Key ion channel subtypes and their neurophysiological roles.

Key ion channel subtypes	Neurophysiological role	Axonal localization	References
Transient Na^+ channels Na_t	Action potential generation; represent majority of Na^+ current	High density at nodes of Ranvier	Ritchie and Rogart (1977), Schwarz et al. (1995), Caldwell et al. (2000)
Persistent Na^+ channels Na_p	Demonstrate incomplete inactivation, modulation of excitability	May reflect differential gating of Na_t channel population	Bostock and Rothwell (1997), Baker and Bostock (1997), Krishnan et al. (2009)
Slow K^+ channels K_s	Outward rectification, limitation of ectopic firing, Reduction of excitability following impulse trains	Highest density at nodes of Ranvier	Baker et al. (1987), Schwarz et al. (1995), Devaux et al. (2004)
Fast K^+ channels K_f	Dampen excitability after action potential generation to prevent re-excitation	High density in juxtaparanode	Chiu and Ritchie (1984), Wang et al. (1993), Rasband et al. (1998)
Hyperpolarization activated cation conductance I_h	Stabilizing membrane potential and excitability; inward rectification	Predominantly expressed at internode	Baker et al. (1987), Pape (1996), Krishnan et al. (2009)
Na^+/K^+ pump	Maintenance of low intracellular Na^+ concentration and membrane potential	Unclear localisation (nodal, paranodal and/or internodal)	Morita et al. (1993), Alberti et al. (2007), Krishnan et al. (2009),
Na^+/Ca^{2+} exchanger	Removal of excess Ca^{2+}; reverse operation can lead to axonal injury and cell death	Axonal membrane; Internode and nodal regions	Steffensen et al. (1997), Persson et al. (2010)

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reviewed in these guidelines (Krishnan et al 2009; Kiernan and Lin, 2012), but direct recordings from unmyelinated axons can be made using microneurography. These recordings have enabled excitability properties to be documented in patients and healthy subjects (Serra et al., 1999; Serra, 2009; Serra et al., 2009). The underlying physiology accessible via axonal excitability techniques will be reviewed in the next section.

2. Physiological basis for changes in excitability

Standardised axonal excitability protocols have been developed to optimise the utility of the technique in the clinical setting. Semi-automated protocols utilising specialised software were initiated at the 1999 Nordic course in clinical axonal electrophysiology as the TROND protocol (Kiernan et al., 2000). Current axonal excitability protocols derived from this initial protocol assess multiple measures of axonal excitability (Table 2). While threshold is a measure of excitability and may be used as a surrogate biomarker of membrane potential, the interpretation of threshold changes may be confounded by a variety of factors, and it is usually necessary to assess multiple threshold parameters to interpret a recorded change in threshold (Baker and Bostock, 1989; Bostock et al., 1994; Mogyoros et al., 1997; Grosskreutz et al., 1999, 2000).

The key elements examined in standardised axonal excitability protocols (Fig. 2) include the stimulus-response curve and strength-duration properties (using test stimuli of different duration), threshold electrotonus and the current/threshold relationship (using subthreshold conditioning stimuli) and the recovery cycle (using suprathreshold stimuli). These properties are reviewed in full in Bostock et al. (1998) and Burke et al. (2001). With the exceptions of the stimulus-response curve and strength-duration properties, manoeuvres are designed to see how axons behave when membrane potential is changed. No individual ion channel is uniquely responsible for a single process, but insights into channel activity can be derived by documenting coherent behaviour across a full battery of tests. In addition, different properties overlap and merge into one another such that changes in an early property may have later effects e.g., the gradual closure of slow K^+ channels is a major factor in the late subexcitable phase of the recovery cycle, but this phase is preceded by a superexcitable phase, and changes in it will alter threshold during the late subexcitable phase (discussed later).

2.1. Strength-duration properties

The strength-duration time constant (τ_{SD}) assesses the relationship between the strength and duration of a stimulus and is related to both active and passive membrane properties (Fig. 2A). The "passive" membrane time constant measures the time for membrane potential to decay in an exponential fashion and this value has been estimated to be 45–50 μs for human nerve, and is similar for motor and sensory axons (Bostock and Rothwell, 1997). However in axons, the injected current will alter membrane potential and thereby the activity of channels active within that voltage range, and this will slow the recovery from a test pulse. Accordingly, the strength-duration time constant in human axons is typically 450–600 μs.

In normal nerve, the major voltage-dependent channels active near resting threshold are persistent Na^+ channels and slow K^+ channels, and both are activated more by membrane depolarization. The only Na^+ channel expressed at the mature mammalian node of Ranvier is thought to be $Na_v1.6$ (Caldwell et al., 2000), but this channel can have two gating modes: transient and persistent. The majority of channels (perhaps 98%) behave in the classical Hodgkin/Huxley manner, opening when depolarized sufficiently, inactivating when held depolarized, and closing when returned to or below resting membrane potential. It is estimated that ~30% channels are inactivated at resting membrane potential (Schwarz et al., 1995). Some channels do not inactivate or do so slowly when held depolarized, and they therefore continue to pass a persistent inward Na^+ current that is de-stabilizing: they depolarize the membrane further and that opens more Na^+ channels, producing greater depolarization. The voltage for activation of channels with persistent gating is depolarized by ~15 mV compared to transient

Table 2
Key excitability variables for median motor and sensory axons.

Motor median nerve excitability			
	29 healthy volunteers Kiernan et al. (2000)	15 healthy volunteers Tomlinson et al. (2010)	105 healthy volunteers McHugh et al. (2011)
SDTC (ms)	0.43	0.42	0.52
TEd peak (%)	69.6	67.3	69.1
TEd 90–100 (%)	45.7	43.1	44.8
TEh 90-100 (%)	−120.8	−115.7	−121.4
S2 accommodation	23.97	24.2	NR
Hyperpolarizing I/V slope	0.375	0.329	0.35
Minimum I/V slope	0.248	0.238	0.25
Resting I/V slope	0.616	0.584	0.59
RRP (ms)	3.14	3.07	3.0
Superexcitability (%)	−25.4	−23.4	−23.6
Subexcitability (%)	14.7	14.4	14.9
Sensory median nerve excitability			
	70 healthy volunteers Lai et al. (2015a)	20 healthy volunteers Matamala et al. (2018)	33 healthy volunteers Sung et al. (2017)
SDTC (ms)	0.617	0.59	0.62
TEd peak (%)	58.6	61.47	59.36
TEd 90–100 (%)	50.4	48.63	49.06
TEh 90-100 (%)	−132.7	−133.9	−145.44
S2 accommodation	8.17	NR	10.3
Hyperpolarizing I/V slope	0.314	0.38	0.39
Minimum I/V slope	0.236	0.25	0.24
Resting I/V slope	0.56	0.56	0.55
RRP (ms)	3.9	3.21	3.44
Superexcitability (%)	−18.7	−15.22	−16.61
Subexcitability (%)	12	8.54	12.39

NR: not reported.

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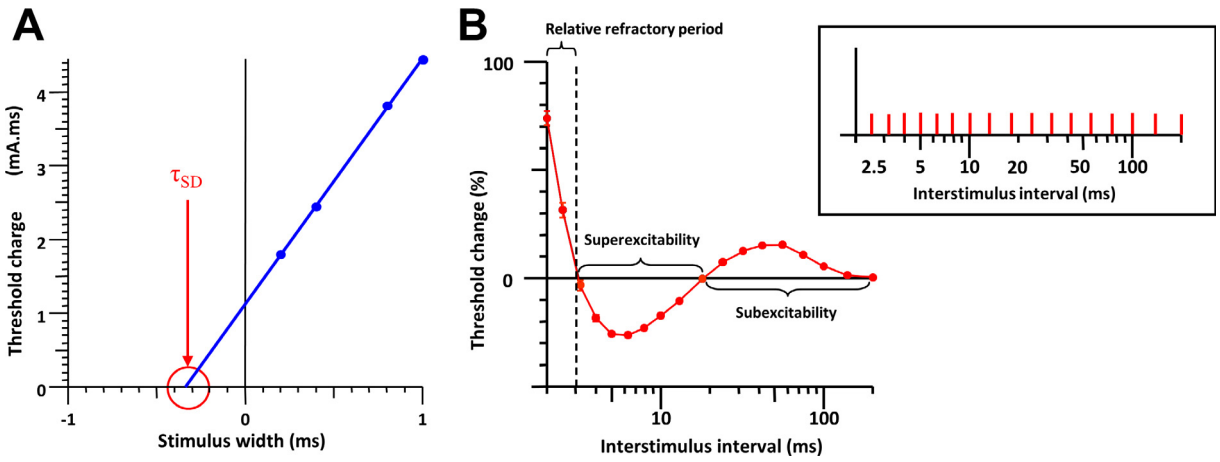

Fig. 2. Excitability parameters. (A) Plot of stimulus width and threshold charge with the strength-duration time constant (τsd) as the negative intercept on the x-axis. (B) Recovery cycle with the relative refractory period, superexcitability and subexcitability depicted on the figure. The timing of conditioning stimuli is depicted in the box to the right.

channels, so that these channels are active at resting membrane potential (and can be "turned off" by a hyperpolarizing test current in the technique of latent addition; Bostock and Rothwell, 1997).

2.2. Recovery cycle

The recovery cycle utilises paired pulses with varying interstimulus intervals (typically between 2 and 200 ms) to assess the recovery of excitability following impulse conduction (Fig. 2B). The components of the recovery cycle overlap, such that changes in one phase will have effects on the size of subsequent phases. 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 (Schwarz et al., 1995). K^+ channels with fast kinetics contribute little because they are largely located in the juxta-paranodal region under the myelin, and there is little access to them over the time course of refractoriness. However there can be more ready access to these channels when the integrity of the paranodal seal is disrupted by disease (e.g., paranodal demyelination; Jankelowitz and Burke, 2013; Garg et al., 2018 or by structural deficiency (e.g., in ALS; Howells et al., 2018a). It is commonly claimed that slow K^+ channels cannot contribute to limiting the refractory period because of their slow opening kinetics, but ~35% of slow K^+ channels are open at rest and can pass current during the action potential (Burke et al., 2009), thereby limiting the action current and promoting repolarization.

In myelinated axons, superexcitability reflects the size of the depolarizing afterpotential. This produces re-excitation of the node by the "back-flow" of current from the internodal membrane through low resistance pathways under and through the myelin sheath (Barrett and Barrett, 1982). The amount of current stored on the internodal membrane varies with membrane potential as paranodal fast K^+ channels are opened or closed. Hyperpolarization will close open fast K^+ channels, increasing the depolarizing afterpotential and the extent of superexcitability. Depolarization will open channels, decreasing the depolarizing afterpotential and thereby the extent of superexcitability. The superexcitable phase normally ends 15–20 ms after the conditioning discharge, but it may last 100 ms when axons are hyperpolarized (see Fig. 7 in Howells et al., 2013).

Late subexcitability is due to axonal hyperpolarization and subsides with the gradual closure of slow K^+ channels opened during the conditioning discharge. Axons begin to become subexcitable at 15 ms, peak subexcitability occurs around 35 ms, and this then decays to resting values at ~100 ms. This time course does not reflect the slow K^+ current, which starts much earlier, but is insufficient to raise threshold until superexcitability has waned. As a result, the degree of subexcitability does not provide a pure measure of slow K^+ channel activity because it is a balance between the decaying slow K^+ current and decaying superexcitability. A measure of slow K^+ channel activity less contaminated by superexcitability can be obtained by recording a recovery cycle using two conditioning stimuli 4 ms apart, and then subtracting the recovery cycle recorded using a single conditioning stimulus (Shibuta et al., 2010a; see Fig. 3 in Matamala et al., 2018).

2.3. Threshold electrotonus

Threshold electrotonus indirectly examines membrane potential changes that occur during long subthreshold current pulses, demonstrating properties of the internodal membrane (Burke et al., 2001). The standard threshold electrotonus protocol introduced by Kiernan et al. (2000) used conditioning polarizing currents that were insufficient to activate axons directly and which lasted 100 ms. However, stronger and longer hyperpolarizing currents, e.g., −100% of threshold applied for 300 ms, are required to see the full extent of accommodation to hyperpolarization (Tomlinson et al., 2010; Howells et al., 2012).

Accommodation to depolarizing currents depends on two types of K^+ channel, 'fast' (Kf) channels of the K_V1 family, and 'slow' (Ks) channels of the K_V7 family. The Kf currents primarily limit the early responses to depolarizing currents (labelled the S1 phase in Fig. 3A) while the Ks currents are responsible for the decline in depolarizing electrotonus after the peak (labelled the S2 phase in Fig. 3A). The separate contributions of these two K^+ channel types to electrotonus was resolved by pharmacological blockade in rat axons (Baker et al., 1987), and is evident in threshold electrotonus of human axons in K^+ channelopathies (Tomlinson et al., 2010, 2012).

Accommodation to hyperpolarization is produced by "hyperpolarization-activated cyclic nucleotide-gated" (HCN) channels which pass the hyperpolarization-activated current I_h. The other voltage-dependent currents are switched off, except for the leak current, an ohmic conductance originally thought not to involve specific channels. With weak hyperpolarization, there is both the closure of channels active at rest (mainly the persistent Na^+ current and the slow K^+ current) and activation of I_h. However when axons are hyperpolarized beyond −60% threshold, it is likely

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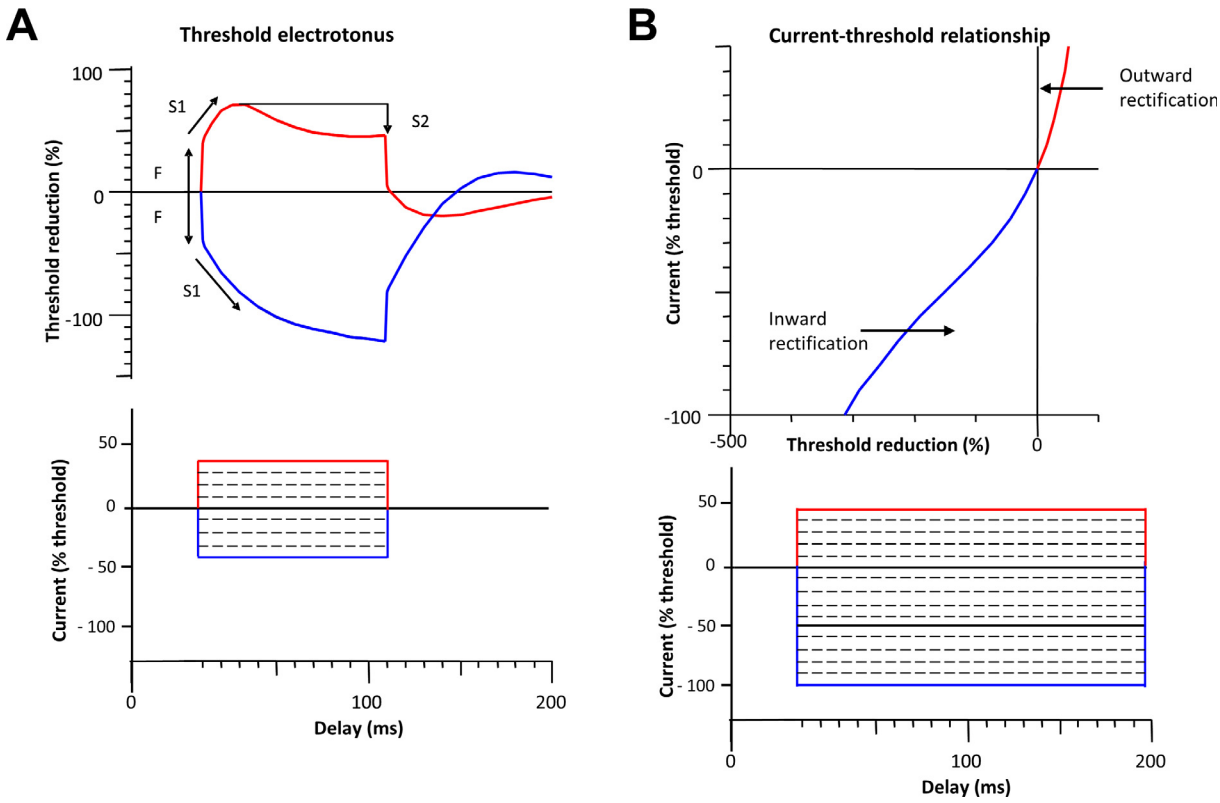

Fig. 3. Excitability parameters. (A) Threshold electrotonus with the initial response to polarization (F phase) followed by slow threshold change (S1 phase) and accommodation to depolarization (S2 phase). Conditioning stimulus current waveforms utilized to generate threshold electrotonus are depicted below. (B) Current-threshold (I/V) relationship plotted with response to hyperpolarizing current in the lower left quadrant and response to depolarizing current in the upper right quadrant. Conditioning stimulus current waveforms utilized to generate the current-threshold relationship are depicted below.

that only I_h and the leak current operate (Howells et al., 2016). Both contribute to the accommodative change in membrane potential. There are four HCN isoforms, and which one or ones are expressed on human peripheral nerve are uncertain. Given the very long time constant of some isoforms, they may be manifest only as a change in membrane potential. There is evidence for human motor axons that an I_h isoform (probably HCN1) is active at resting membrane potential and contributes to the lower threshold of the first recruited axons (Trevillion et al., 2010). When the hyperpolarizing current ends, there is a slowly developing long-lasting decrease in threshold which is due to gradual closure of HCN channels and re-activation of persistent Na^+ currents.

For valid threshold electrotonus, it is important to check that the depolarizing (and hyperpolarizing) currents are subthreshold. If axons are hyperexcitable or depolarized, the conditioning depolarizing current may cause them to discharge even when the strength of the current is only 40% of threshold (Trevillion et al., 2007). This occurs rarely with healthy motor axons, but frequently with healthy sensory axons (Burke et al., 2007). When there is a discontinuity or "notch" in the development of the peak depolarizing change in threshold, it is advised to inspect the raw traces looking for inadvertent discharge in response to the supposedly subthreshold depolarization. The traces with this contamination cannot be used in modelling and, when present, additional threshold electrotonus curves should be recorded using weaker depolarizing currents.

Compared to other measures, there is marked variability in the threshold electrotonus curves to hyperpolarizing currents. This variability appears to be subject-specific: the between-subject variability is much greater than the within-subject variability (Tomlinson et al., 2010; Howells et al., 2013), and this is explicable by differences in channel gating, specifically the voltage for half-activation of a single type of HCN channel (Jankelowitz et al., 2007; Howells et al., 2012). Similarly, the well-documented difference in I_h in sensory and motor axons (Bostock et al., 1994; Lin et al., 2002) is probably not due solely to a difference in expression of I_h. The difference in expression accounts for only some 39% of the difference in I_h, while the rest can be accounted for by a difference in channel gating (Howells et al., 2012). The important message for future studies is that changes in channel activity do not necessarily reflect changes in the expression of the channel: they could result from changes in the gating of a similar number of channels.

2.4. Current-threshold relationship

To explore the accommodative properties axons better, the original Trond protocol incorporated the current-threshold relationship, as an analogue of a current-voltage (I/V) relationship (Fig. 3B). Hyperpolarizing and depolarizing currents lasting 200 ms are utilised and the strength of the injected current is stepped from −100% (hyperpolarizing) to +50% (depolarizing). The effect on membrane potential is indicated by the change in threshold at the end of the injected current. The subsequent 16 measurements (10 hyperpolarizing, zero, 5 depolarizing) form the current-threshold relationship, effectively the "I/V curve", which enables quantification of the resting input conductance and inwardly and outwardly rectifying conductances (Kiernan and Bostock, 2000; Kiernan et al., 2000).

The resting I/V slope (the slope of the I/V curve immediately above and below zero current injection) is a threshold analogue of the resting input conductance, and will be affected by channels open at resting membrane potential. The steepness of the curve in the depolarizing and hyperpolarizing directions reflects inward

and outward rectification, i.e., the extent to which the axons have accommodated to the change in membrane potential – the smaller the change in threshold for an injected current, the greater the accommodation. In the depolarising direction, the accommodation is due to K^+ currents; in the hyperpolarizing direction it is due to I_h. These accommodative changes are best seen in the I/V slope curves, where the slope of the I/V curve is plotted against the change in threshold.

2.5. Latent addition

Latent addition using automatic threshold tracking is mainly used to study persistent Na^+ currents at the node of Ranvier (Bostock and Rothwell, 1997; Kuwabara et al., 2006; Misawa et al., 2006; Kuwabara and Misawa, 2008). Although strength-duration time constant is also used as a surrogate measure of persistent Na^+ currents, it is affected by passive membrane properties. Latent addition can evaluate these two factors separately, and this is the major advantage of this technique (Kuwabara and Misawa, 2008). In brief, latent addition utilises short stimuli (60 μs), with hyperpolarizing conditioning stimuli set to −90% of threshold and a variable conditioning-test interval. The threshold recovery after hyperpolarization is represented by the sum of two exponentials - the fast component reflecting passive membrane properties and the slow component indicating persistent Na^+ conductances (Bostock and Rothwell, 1997).

2.6. Unmyelinated axons

Unmyelinated axons cannot be studied directly in routine conduction studies and the techniques and excitability properties described earlier are not present or have a different basis. However they subserve important sensations, warranting a brief review of their properties.

Within peripheral nerve, axons with diameters of more than 1 μm tend to be myelinated, while axons with diameters of less than 1 μm tend to be unmyelinated (Waxman and Bennett, 1972). These unmyelinated axons, termed C-fibres, arise from small-diameter neurons in the dorsal root ganglion (Waddell and Lawson, 1990). Within peripheral nerves and roots, the unmyelinated axons run either singly, or in small groups in which they can be juxtaposed, within furrows of investing but non-myelinating Schwann cells (Ochoa and Mair, 1969). In contrast to myelinated axons which conduct action potentials in a saltatory manner, conduction along unmyelinated axons proceeds along the fibre in a continuous manner. The continuous mode of conduction in unmyelinated axons is partly a consequence of the absence of low-capacitance, high-resistance myelin sheaths, and appears to be supported by a relatively homogeneous distribution of Na^+ channels along the axonal trajectory. In terms of sodium channels, $Na_v1.1$ (Black et al., 1996), $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ (Persson et al., 2010) and $Na_v1.6$ (Black et al., 2002) have been detected immunocytochemically along the shafts of unmyelinated PNS axons, although these studies do not differentiate between intracytoplasmic channels and functional channels inserted into the axonal membrane. The overall density of Na^+ channels within the membrane of unmyelinated axons has been roughly estimated at about 10^2 per μm^2 (Pellegrino et al., 1984). Conduction velocity along unmyelinated peripheral nerve axons is generally less than 1–2 m/s, slower than in myelinated axons of the same diameter. Because the surrounding Schwann cells do not seal off unmyelinated axons from the surrounding extracellular milieu, these axons can be influenced by changes in the ionic microenvironment.

Unmyelinated axons (C-fibres) subserve functions such as temperature and pain sensation. Their dysfunction is not necessarily accompanied by objective signs on neurological examination, such as the hyporeflexia that accompanies dysfunction of larger myelinated axons. This underscores the importance of electrophysiological assessment by microneurography (e.g. Serra et al., 1999; Serra, 2009; Serra et al., 2009).

3. Hardware and software requirements

Threshold tracking typically utilises QTracS software (© UCL Institute of Neurology, London, UK, available from Digitimer Ltd at www.Digitimer.com), a nerve excitability stimulus control, acquisition and data analysis software package (Fig. 4) with a number of relevant features:

- a choice of tracking modes for determining the step size, from fixed step (e.g., 2%) to proportional step (i.e., step size proportional to difference between actual and target response;
- the ability to cycle between different stimulus channels, each with different conditioning and test stimulus combinations, keeping separate records of the threshold and tracking history for each;
- the option to fix the amplitude of the conditioning stimulus on one channel to a specified fraction of the test stimulus on another channel, used, e.g., for latent addition and threshold electrotonus;
- the option to subtract the response on one channel from that on another, before comparing the response with the target response (e.g., in measurements of the recovery cycle when the conditioning-test interval is short, and the response to the conditioning stimulus overlaps with the response to the test stimulus); and
- the option to increment one of a number of time intervals (e.g., the conditioning–test interval), either in regular steps or in a predetermined irregular sequence, and either after a fixed number of test stimuli or only when threshold has been determined to a specified accuracy.

Axonal excitability data generated by the TROND protocols can be analysed directly in QtracP (© UCL Institute of Neurology, London, UK, available from Digitimer Ltd at www.Digitimer.com), which creates to special text files (.MEM) for further analysis. Complete nerve excitability data from a group of MEM files indexed by a MEF file can be plotted or exported to an Excel file.

3.1. Stimulator

The DS5 isolated bipolar stimulator developed by Digitimer Ltd allows computerised control of stimulus amplitude and timing parameters and has a maximal constant current output of ±50 mA. The DS5 is controlled by an analogue voltage input which it translates into an isolated constant-current stimulus (up to ±50 mA), precisely replicating the shape of the input waveform.

3.2. Data acquisition system

The National Instruments (NI) USB-6341 with BNC terminals (Part 782251-01) is suitable for threshold tracking. The QtracS driver sets up the NI cards with an input range of ± 10 V and 8 differential inputs (AI0-7). NI-DAQmx software must be version 7.5 or later. If speed is critical, PCI boards are faster than USB boards (reference source: Qtrac manual 2016 version).

3.3. Amplifier

For axonal excitability testing, compound action potentials have to be amplified (×100 to ×20k) and bandpass filtered (3 Hz to

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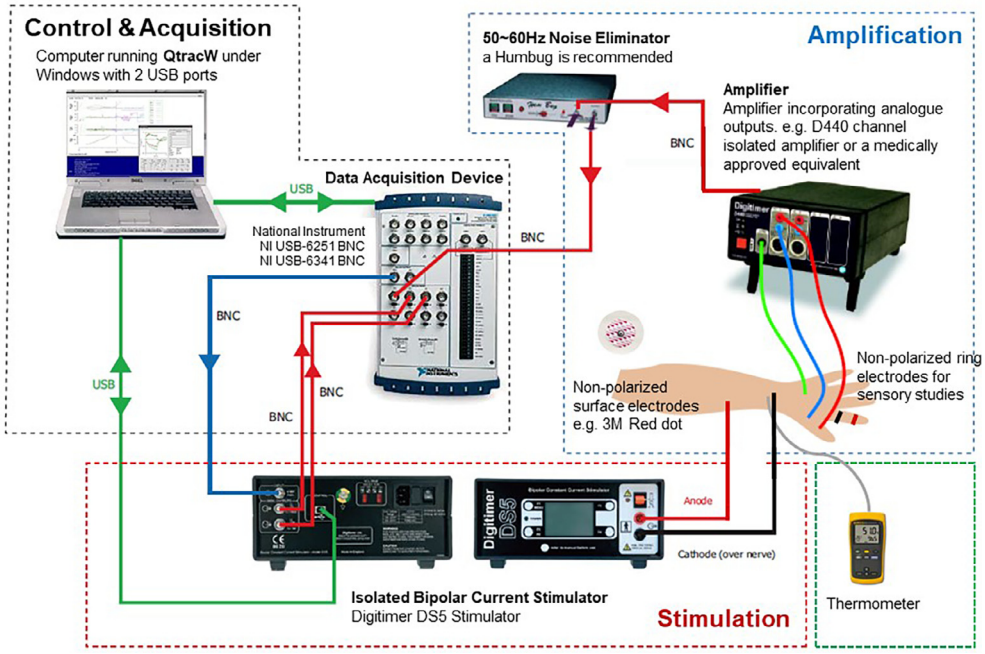

Fig. 4. Setup for testing human axonal excitability**.** Depiction of testing setup demonstrating connectivity of computer, data acquisition board, stimulator, amplification devices and patient.

3 kHz). The amplifier should have analogue signal outputs making it compatible with commercial data acquisition systems. The D440 amplifier was designed by Digitimer specifically for nerve excitability studies on both sensory and motor nerves. It has good low-noise performance suitable for recording submaximal sensory nerve action potentials (SNAPs). In addition, Howells et al. (2018b) have developed a bespoke amplifier to be able to track small SNAPs of only a few microvolts amplitude.

4. Important controls

4.1. General considerations

Axonal excitability techniques have primarily been utilised to identify pathophysiological mechanisms, rather than to serve as diagnostic tools. As such, the determination of diagnostic cut-off values and estimates of diagnostic accuracy and precision (as per the STARD criteria) would be required before axonal excitability studies would be considered implementable for routine diagnostic purposes. However, excitability studies are useful in the clinical setting, in the context of providing pathophysiological insights that are complementary to other electrodiagnostic tests and clinical findings.

At an individual patient level, axonal excitability studies can produce results that distinguish individual patients from healthy controls (e.g. including oxaliplatin-induced neurotoxicity in sensory axons Park et al., 2009b; multifocal motor neuropathy in affected motor axons Kiernan et al., 2002a; hereditary neuropathy with liability to pressure palsies Jankelowitz and Burke, 2013; Farrar et al., 2014; episodic ataxia type 1 Tomlinson et al., 2010; Tan et al., 2013; renal neuropathy Kiernan et al., 2002b; Krishnan et al., 2005b). The number of control subjects needed varies across disorders. Because excitability studies provide information about axonal membrane function through a number of parameters, it is not possible to define a standard of abnormality that would be appropriate across multiple disorders and hence to define a minimum sample size of healthy controls that would be suitable across conditions.

However, of note, routine electrodiagnostic values are not always distinct from healthy controls and individual patients, and reference values solely provide information on the probability of an individual's results being abnormal (Campbell and Robinson, 1993). Taken in total, excitability studies provide evidence to aid diagnosis and distinguish conditions from disease mimics, however should be utilized in the context of clinical findings.

Regarding factors which may be important in terms of influencing excitability recordings, there is evidence that potassium and sodium blood levels (Kuwabara et al., 2007; Boërio et al., 2014; Park et al., 2014), ion channel active medications and temperature are particularly relevant. A number of drugs have purported effects on ion channel function, and may therefore influence peripheral nerve excitability, however exact effects remain unclear. Of these riluzole (Vucic et al., 2013), mexilitene (Kuwabara et al., 2005), lidocaine (Moldovan et al., 2014) and flupirtine (Fleckenstein et al., 2013) have been demonstrated to affect peripheral nerve excitability.

4.2. Noise elimination

Signals recorded using biological or other high impedance devices are often contaminated with 50 Hz noise. This electrical interference may be extremely difficult to remove without altering the original signal embedded within the noise, though attention to grounding preparation and appropriate shielding can reduce or eliminate electrical interference. The Hum Bug (Quest Scientific Instruments, North Vancouver, Canada) can eliminate 50 Hz noise from analogue signals. It is equally effective at removing noise associated with inadequate grounding, ground loops, and electrical pick up. The Hum Bug is not a filter, it does not create phase delays, amplitude errors, DC shifts, or waveform distortion. It is connected between preamplifier and data acquisition device. Hum Bug is particularly useful when testing sensory nerve excitability, because

Please cite this article as: M. C. Kiernan, H. Bostock, S. B. Park et al., Measurement of axonal excitability: Consensus guidelines, Clinical Neurophysiology, https://doi.org/10.1016/j.clinph.2019.07.023

the maximal compound sensory action potential is small and the signal-to-noise ratio is low.

***Assessment of temperature*:** Skin temperature should be measured at the site of stimulation throughout the test, and kept stable, greater than 32 °C. The relative refractory period is very sensitive to temperature and may be prolonged by cooling because of slowed channel kinetics at lower temperatures (Stys and Ashby, 1990; Burke et al., 1999; Kiernan et al., 2001b; Mogyoros et al., 2000), and axonal depolarization due to an effect of cooling on Na^+/K^+-pump activity (Franssen et al., 2010; Kovalchuk et al., 2018). Hyperthermia may challenge the ability of diseased axons to transmit action potentials faithfully (Howells et al., 2013): there were clear changes in excitability during hyperthermia, with reduced superexcitability following an action potential, faster accommodation to long-lasting depolarization and reduced accommodation to hyperpolarization.

4.3. Methodological and practical considerations

Stimulation

1. Select appropriate stimulating electrodes. If the current density is greater than 2 mA rms/cm^2, the patient might be at risk of burns at the stimulation site.
2. Do not connect the stimulator to patients and high-frequency surgical equipment or microwaves concurrently. Doing so could cause burns, and the output of stimulator will be affected.
3. The stimulator should not be operated in strong magnetic fields, such as in an MRI scanning room.
4. Stimulators should not be applied to patients thoracically or trans-thoracically because this increases the risk of cardiac fibrillation.
5. Patients with cardiac pacemaker should not undergo the nerve excitability test without specialist's medical advice.

Recording

Axonal excitability studies require stable tracking of stimulus current over the course of the test – accordingly threshold and unconditioned response should be monitored to ensure that major changes do not occur. Manoeuvers such as ischaemia or activity may reduce the amplitude of the maximal response, necessitating continuous monitoring of responses during assessment (Kiernan et al., 1997; Mogyoros et al., 1997; Lin et al., 2002). Identification of the optimal stimulation site in terms of lowest threshold is important, as is appropriate skin preparation to reduce impedance.

Provided that conduction is maintained, there is no theoretical limit regarding the number of axons contributing to the recorded response, because the tracking mode can be adjusted to track the activity of single motor units. Stimulation rates should be kept slower than 3 Hz, to avoid cumulative effects. When length-dependent involvement is suspected it could be relevant to study excitability at multiple sites along the same nerve. In the upper limb, proximal stimulation along the median nerve is typically carried out at elbow with recording over the forearm flexors (such as flexor carpi radialis). In other studies, recording has been carried out also over abductor pollicis brevis to ensure that the same axons were studied (Moldovan et al., 2014). In the lower limb, excitability of the peroneal nerve at the fibular neck has been tested by recording from tibialis anterior (Kuwabara et al., 2001, 2002a), and a length-dependent gradient was established by recording from tibialis anterior, extensor digitorum brevis, and abductor hallucis (Krishnan et al. 2004; 2005a, 2006a; Kuwabara et al., 2001; Boland et al., 2009). It has been suggested that differences in biophysical properties could contribute to differences in susceptibility to neuropathy (Van der Heyden et al., 2013; Lin et al., 2000). Specialized recordings have assessed facial nerve branches (Eviston and Krishnan, 2016) as well as a preliminary report on the spinal accessory nerve in severe demyelinating CMT when median nerve studies were not possible (Krarup et al., 2017; Pisciotta et al., 2018).

5. Interpretation of clinical results: the role of mathematical modelling

Nerve excitability properties depend on the complex interactions between nodal and internodal ion channels and ion pumps, passive membrane components, and the intracellular and extracellular constituents of the nerve. Because of this complexity, it can be very difficult to predict the effects of any pathological change, and even more difficult to deduce the biophysical basis of an abnormal nerve excitability recording. However, a simplified mathematical model has been generated that accounts quite well for the common nerve excitability measurements made on normal human peripheral motor axons, and in certain favourable situations this can be helpful in the interpretation of abnormal recordings (for examples see references Kiernan et al., 2005a; Howells et al., 2018a).

If considering the use of modelling to aid interpretation of recordings from a group of patients, it is important to bear in mind that:

(a) nerve excitability recordings depend on the properties of multiple nerve fibres at a single site, so modelling is likely to be helpful only if the axons are uniformly affected, across both the width and length of the nerve;
(b) patient excitability measurements need to be consistently and substantially abnormal (e.g. with no overlap in some parameters between interquartile ranges of patient and control groups) to provide enough information to model the difference;
(c) 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;
(d) there are small but significant changes in nerve excitability with age and temperature (but not sex, Casanova et al., 2014), so that it is desirable to first assemble a control set of recordings which match in these variables. There are also age-related changes that occur with nerve maturation in children (Farrar et al., 2013). Mathematical modelling of nerve excitability abnormalities has been applied to a range of neurological conditions, and some examples will be highlighted in relation to their utility in interpreting patient recordings in the clinical studies section that follows.

6. Clinical studies

6.1. Immune-mediated neuropathies

In acquired neuropathies, paranodal or internodal demyelination results in exposure of ion channels, and changes in myelin resistance, whereas immune attack on the nodal axolemma can impair sodium channel function at the node. The structural changes can cause complex alterations in axonal properties. Most excitability studies have compared data from patient groups with normals or disease controls. Each type of immune-mediated neuropathy tends to show characteristic findings, according to its pathophysiology, and nerve excitability studies can provide useful information about the pathology, diagnosis, and treatment. Specif-

Please cite this article as: M. C. Kiernan, H. Bostock, S. B. Park et al., Measurement of axonal excitability: Consensus guidelines, Clinical Neurophysiology, https://doi.org/10.1016/j.clinph.2019.07.023

ically, the response to therapy can be objectively assessed through paired studies pre- and post-treatment (such as before and after infusion with intravenous immunoglobulin).

In immune-mediated neuropathies such as Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyneuropathy (CIDP), the distal nerve terminals and nerve roots, where the blood-nerve barrier is anatomically deficient, are preferentially affected by the immune attack. Therefore, excitability indices may be normal at the tested site such as the median nerve at the wrist. This is the case in the early phase of GBS, but not in CIDP.

GBS is currently classified as acute inflammatory demyelinating polyneuropathy (AIDP), the classical demyelinating form, or acute motor (and sensory) axonal neuropathy (AMAN/AMSAN), an axonal variant. The pathology in the two subtypes is different, and so too are the excitability changes. Three studies have compared excitability properties in AIDP and AMAN/AMSAN (Kuwabara et al., 2002a, 2003; Pyun et al., 2017). All excitability indices (τ_{SD}, threshold electrotonus, recovery cycle, and current/threshold relationship) were normal, findings explained by demyelinating lesions selectively affecting the nerve terminals and roots, sparing the wrist portion where testing was performed.

In contrast, patients with AMAN/AMSAN have high refractoriness and an impaired refractory period of transmission. When pairs of stimuli were delivered at short intervals (2 ms), the second impulse was blocked due to inactivation of nodal sodium channels by the conditioning discharge. Among excitability indices, this was the single abnormality, suggesting that nodal sodium channels were impaired by anti-ganglioside antibodies. Specifically, ganglioside GM_1 and GD_{1a} constitute a lipid raft of the axonal membrane, and deposition of antibodies against these structures impairs nodal structures such as sodium channels. As such, axonal excitability testing can be recommended in axonal GBS patients to assess sodium channel function and the safety factor for impulse transmission in the distal motor nerve terminals.

In contrast, for patients diagnosed with CIDP, the median nerve at the wrist is commonly affected by demyelination. Segmental demyelination reduces myelin resistance and exposes ion channels. Two studies reported multiple abnormalities in excitability measurements for typical CIDP (Cappelen-Smith et al., 2001; Sung et al., 2004). The most striking changes were observed in threshold electrotonus, with a significant "fanning-out" (i.e., greater threshold changes to the long conditioning depolarizing and hyperpolarizing currents). Similarly, Sung et al. (2004) found that 48% of CIDP patients had abnormal threshold electrotonus that correlated with disease duration and severity, with a poorer response to immunotherapies. As such, threshold electrotonus may be useful to detect demyelination to provide new insights into the pathophysiology and distribution patterns of demyelination in CIDP (Garg et al., 2017).

Subsequently, Lin et al. (2011) established that many of the excitability indices, such as τ_{SD}, threshold electrotonus, and superexcitability, changed immediately after the start of intravenous immunoglobulin therapy, a rapid response to the modulatory effects of immunoglobulin treatment. Sung et al. (2014) reported that by measuring superexcitability, subexcitability and hyperpolarizing threshold electrotonus (10–20 ms), patients with AIDP and acute-onset CIDP could be clearly separated into two non-overlapping groups. Nerve excitability techniques may be useful in distinguishing acute-onset CIDP from AIDP at the initial stage, leading to more directed and appropriate treatment.

While there may remain some debate about the nature of multifocal motor neuropathy (MMN) and specifically whether the condition represents a demyelinating or axonal disease, a number of studies have addressed the pathophysiology of MMN and the cause of focal conduction block. Axonal properties remote from foci of conduction block are normal, findings that support the contention that MMN is not a generalized disease with a focal presentation (Cappelen-Smith et al., 2002). However, excitability studies have also been undertaken in MMN patients just distal to the site of conduction block in affected nerves, with strikingly abnormal findings (Fig. 5A; Kiernan et al., 2002a; Garg et al., 2017). The most significant alterations in excitability properties in the MMN patients were: (a) the reduction in minimum slope of the current/threshold ('I/V') relationship (indicating a reduction in input conductance); (b) the "fanning-out" of threshold electrotonus; and (c) the increase in superexcitability recorded during the recovery cycle. All these excitability parameters, highly abnormal in the MMN patients, depend on the resting conductance of the paranodal and internodal axon membrane. As a consequence of these findings, it was hypothesised that the nerve distal to the site of conduction block in MMN patients was behaving as if hyperpolarized, most closely resembling a post-ischaemic nerve.

6.2. Hereditary neuropathies

There have been a range of excitability approaches across a wide spectrum of hereditary neuropathy. In terms of hereditary sensorimotor neuropathy, 4 studies undertaken in Charcot Marie Tooth Disease CMT1A and CMTX (Nodera et al., 2004; Sung et al., 2004; Jankelowitz and Burke, 2013; Liang et al., 2014) identified an increase in stimulation threshold associated with 'fanning-out" of threshold electrotonus associated with subtle changes in the recovery cycle consistent with changes in passive cable properties. Nevertheless, as compared to CMTX, CMT1A showed higher threshold currents, smaller changes in TEh (90–100 ms) and a reduced superexcitability. These findings were taken to indicate that in CMT there were also changes in active membrane properties which could differentiate between different CMT mutations at group level, as also supported by preliminary reports in CMT1B where the culprit relates to mutation in the MPZ gene (Krarup et al., 2017; Pisciotta et al., 2018).

In relation to the condition hereditary neuropathy with liability to pressure palsies (HNPP), results from 3 excitability studies (Jankelowitz and Burke, 2013; Farrar et al., 2014; Liang et al., 2014) indicated that both motor and sensory excitability tests were abnormal in HNPP. The abnormalities included an increased simulation threshold and "fanning-out" of the threshold electrotonus. Although these deviations were not specific to HNPP, the extent of the increase in threshold during hyperpolarization as well as the heterogeneity of abnormalities across stimulation sites provided a mechanism to differentiate HNPP from CMT1A at group level.

Studies on amyloid neuropathy remain limited, with a single report on threshold tracking undertaken in 10 patients with primary amyloidosis confirmed by nerve conduction studies (Hafner et al., 2015; Lai et al., 2015). The study identified decreased excitability of sensory fibres but otherwise there were no further abnormalities in threshold tracking of motor or sensory fibres.

Threshold tracking in patients diagnosed with Fabry disease harbouring an X-linked α-galactosidase mutation identified reduced threshold reduction to depolarizing electrotonus and reduced superexcitability. These findings were determined to be consistent with nerve fibre depolarization possibly due to ischemia caused by glycosphingolipid accumulation in vascular endothelial cells (Tan et al., 2005). "Fanning-in" of motor fibres was confirmed in another study that also identified a steeper relationship of the hyperpolarizing current/threshold (I/V) relationship indicating increased I_h (HCN) function that was correlated with clinical neuropathy scores (Geevasinga et al., 2012). This upregulation of I_h was suggested to be associated with features of pain as experienced by patients diagnosed with the disorder.

Please cite this article as: M. C. Kiernan, H. Bostock, S. B. Park et al., Measurement of axonal excitability: Consensus guidelines, Clinical Neurophysiology, https://doi.org/10.1016/j.clinph.2019.07.023

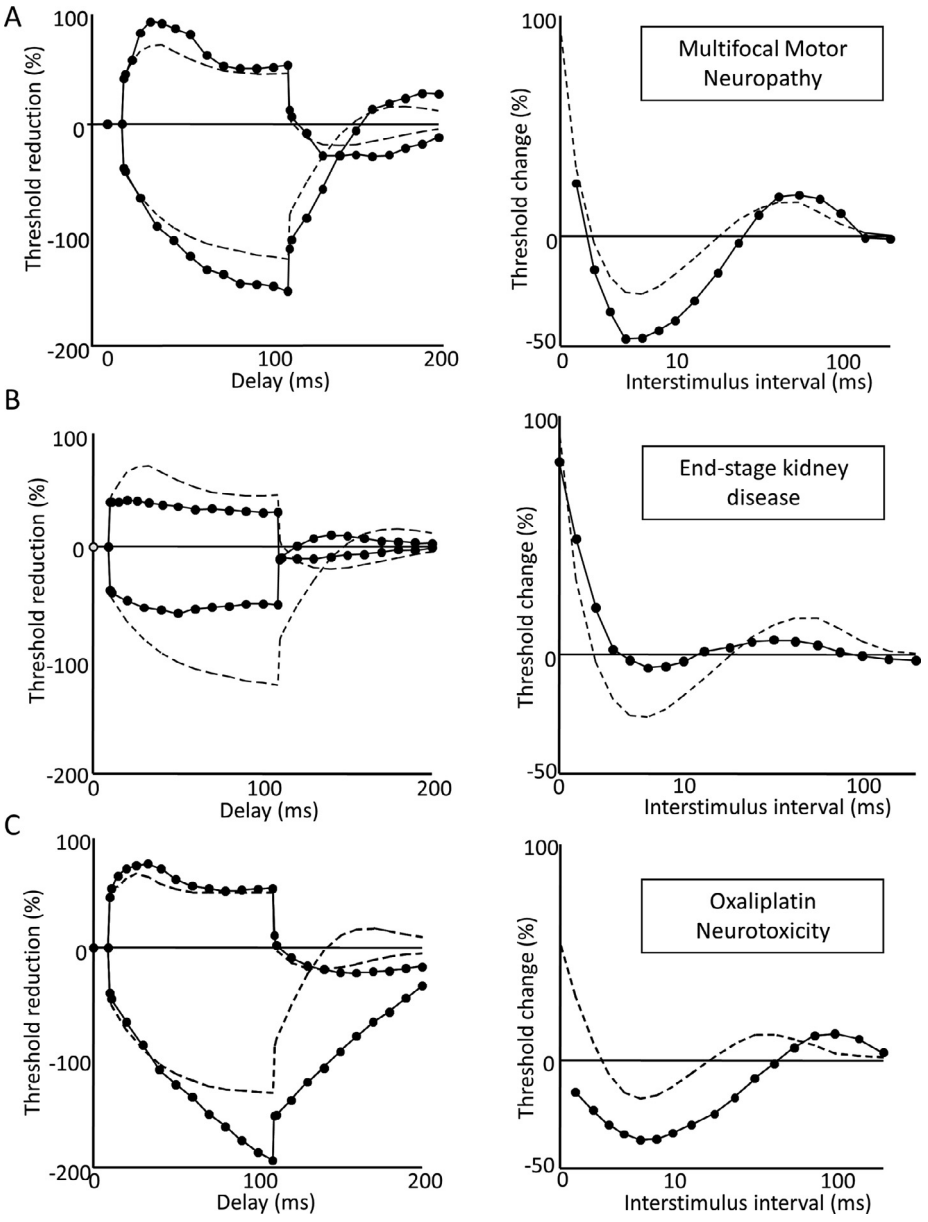

Fig. 5. Alterations in axonal excitability in the clinical setting. Left panels depict threshold electrotonus (±40% threshold) and right panels depict recovery cycle recordings. Dashed lines represent healthy control values and black dots represent patient values. (A) Multifocal motor neuropathy: demonstrating threshold increase in threshold electrotonus and increased superexcitability in recovery cycle in affected motor median nerve, (B) End-stage kidney disease: demonstrating threshold reduction in threshold electrotonus and reduction of superexcitability in recovery cycle in motor median nerve, (C) Oxaliplatin neurotoxicity: demonstrating threshold increase in hyperpolarizing threshold electrotonus and reduced refractoriness in recovery cycle in sensory median nerve.

Turning attention to excitability approaches to explore the pathophysiology of porphyric neuropathy (Lin et al., 2008, 2011), patients diagnosed with latent acute intermittent porphyria (AIP) but without superadded neuropathy, threshold tracking showed greater mean threshold changes during hyperpolarizing threshold electrotonus and a decrease of the current/threshold (I/V) slope during hyperpolarization. Mathematical modeling of these recordings showed that such changes could be explained through reduction in I_h (HCN) current. Separately, a patient who was diagnosed with acute neuropathy during an AIP attack showed changes consistent with axonal depolarization, which was interpreted as evidence of failure of the Na^+/K^+-pump within the axonal membrane. The interpretation of reduced I_h in porphyria suggested an intracellular energy failure affecting the function or level of cAMP (Lin et al., 2008). Thus, threshold tracking can be useful in acute neuropathy in AIP as the changes appear to differentiate from axonal GBS (Kuwabara et al., 2002c).

6.3. Epilepsy

Excitability testing in patients diagnosed with epilepsy linked to known genetic mutations has a role in research and has demonstrated that channelopathies affecting the central nervous system may also express changes in peripheral nerve function, where these channels are similarly co-expressed (Tomlinson et al., 2018). Examples include mutations in *SCN1A* and *SCN2A* on chromosome 2q24 that encode for α-subunits of $Na_v1.1$ (SCN1A) and $Na_v1.2$ (SCN2A), implicated in a variety of genetic epilepsy syndromes (Kullmann, 2010; Meisler et al., 2010), although these conditions have yet to be examined using excitability techniques.

Mutations in the *SCN1B* on chromosome 19, encoding the auxiliary β1-subunit reduces Na^+-channel trafficking and has been linked to $GEFS^+$. Threshold tracking of peripheral nerve in patients with GEFS + due to a mutation in *SCN1B* implicating the β1-subunit in $Na_V1.6$ channel function in myelinated fibres (Kiernan et al., 2005b) established reduced Na^+ current based on a shorter τ_{SD}, less refractoriness and larger fanning-out during hyperpolarizing threshold electrotonus. These findings were similar to results in patients experiencing tetrodotoxin poisoning, with reduction in the function of axonal Na^+ channels (Kiernan et al., 2005a).

Threshold tracking in patients diagnosed with benign familial neonatal seizures (BFNS) due to a mutation in *KCNQ2* (Tomlinson et al., 2012) established reduced accommodation during depolarizing threshold electrotonus, consistent with reduced slow K^+-current as had previously been demonstrated in animal models (Schwarz et al., 2006). Interestingly these patients did not demonstrate peripheral nerve hyperexcitability as has otherwise been found in patients with disturbed $K_V7.2$ channels (Wuttke et al., 2007; Maljevic et al., 2010) which could be explained by an adaptive increase in fast potassium current.

Excitability studies have also been undertaken in the related condition episodic ataxia types 1 and 2 (EA1, EA2 Tomlinson et al., 2010, 2016). Patients with EA1 and neuromyotonia linked to mutation in $K_V1.1$ identified abnormalities consistent with markedly decreased threshold during depolarizing threshold electrotonus and during the superexcitable period. In contrast, changes in excitability in EA2 patients could not be accounted for by changes in a single channel, and mathematical modelling was more suggestive of involvement of Schwann cells and opening of the Barrett-Barrett resistance.

6.4. Neurodegeneration

Excitability techniques have provided insights into the pathophysiological mechanisms underlying the development of neurodegeneration and clinical features of amyotrophic lateral sclerosis (ALS) and related neuromuscular disorders. Prolonged τ_{SD}, increased superexcitability and abnormalities of threshold electrotonus have been identified as a signature of axonal dysfunction in ALS across multiple studies. These changes have been linked to changes in ion channel function, particularly increased Na^+ and decreased axonal K^+ conductances, a profile which may lead to neurodegeneration and contribute to symptoms such as fasciculations and muscle cramps (de Carvalho et al., 2017; Tsugawa et al., 2018). Increased τ_{SD} has been identified in sporadic ALS (Mogyoros et al., 1998; Kanai et al., 2006; Vucic and Kiernan, 2006; Vucic et al., 2007), as well as in atypical ALS phenotypes, such as the flail arm variant (Vucic and Kiernan, 2007a), and in familial forms of ALS linked to superoxide dismutase-1 (SOD1) and c9orf72 (Vucic et al., 2008; Vucic and Kiernan, 2010; Geevasinga et al., 2015). Prolongation of τ_{SD} in ALS has been linked to upregulation of persistent Na^+ conductances (Kuo et al., 2005), with additional contribution from reduced fast and slow K^+ channel conductances producing depolarization (Vucic and Kiernan, 2006). Interestingly, increased τ_{SD} has been linked to shorter survival in ALS (Kanai et al., 2012), further suggesting that axonal hyperexcitability is pathological important.

In combination with these abnormalities in τ_{SD}, changes in threshold electrotonus and recovery cycle have been identified in sporadic ALS. Increased threshold changes in depolarizing and hyperpolarizing subthreshold currents (a "fanned-out" appearance) have been identified in some studies in ALS (Noto et al., 2011). In contrast, other studies have documented changes in depolarizing threshold electrotonus only (Bostock et al., 1995; Vucic and Kiernan, 2007a). Threshold electrotonus changes in ALS increase with disease progression, associated with axonal degeneration (Cheah et al., 2012). In addition, recovery cycle abnormalities have also been reported in sporadic ALS – with an increase in superexcitability identified in some studies (Vucic and Kiernan, 2006). However, there is heterogeneity in excitability findings, potentially reflecting differences in disease progression. Insights into this variability have been provided by axonal excitability studies undertaken in single motor units in ALS patients (Howells et al., 2018a). A pattern of abnormal excitability was identified involving increased threshold change in depolarizing and hyperpolarizing threshold electrotonus, increased superxcitability and reduced inward and outward rectification. These changes were most pronounced in more severely affected motor units and the extent of changes correlated to strength and fine motor function. Mathematical modelling indicated that a reduction in the expression of ion channels globally explained these findings, potentially linked to failures in axoplasmic transport and delivery of essential proteins from the motor neuron.

Excitability studies have also been undertaken across a range of neurodegenerative disorders, some presenting as a mimic disease of ALS. For instance, Kennedy's disease (spinobulbomuscular atrophy), a slowly progressive inherited neurodegenerative disorder of motor and sensory neurons secondary to increased expansion of the polymorphic cytosine-adenine-guanine (CAG) repeat sequence in the androgen receptor gene on the X chromosome, at Xq11-12, may present as a mimic to ALS (Kennedy et al., 1968). Axonal excitability studies in Kennedy's disease have demonstrated prolonged τ_{SD}, increased depolarizing threshold electrotonus and increased hyperpolarizing current (Vucic and Kiernan, 2007b). Changes in τ_{SD} occurred early in Kennedy's disease and were linked to fasciculation frequency, suggesting that increased persistent Na^+ conductance was responsible for fasciculations, and potentially neurodegeneration. Axonal excitability studies have also identified altered voltage dependence of HCN channels in patients with the disease mimic benign cramp fasciculation syndrome (Czesnik et al., 2015).

From the perspective of paediatric neurodegenerative disease, spinal muscular atrophy (SMA) is a disorder of the spinal motor neurons clinically characterised by development of progressive muscle weakness and atrophy (Farrar et al., 2011). While axonal excitability studies in less severely affected SMA patients identified reduction in motor amplitudes without significant changes in excitability (Farrar et al., 2011), a suite of excitability changes occurred in more severely affected SMA patients incorporating prolonged τ_{SD}, increased depolarizing TE and steepening of early hyperpolarizing TE. These excitability changes were correlated with disease severity and linked via mathematical modelling to reduced internodal length, reduced internodal fast K^+ currents and increased nodal K^+ conductances.

Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominant CAG trinucleotide expansion on chromosome 14 with variable presentation depending on the number of repeats. The disease is characterized by progressive ataxia, peripheral neuropathy, ophthalmoplegia, motor neuron loss, Parkinsonism and spasticity (Schöls et al., 1996). Threshold tracking was carried out in patients with Machado-Joseph disease to investigate the complaints of severe cramps in the lower extremities (Kanai et al., 2003; Farrar et al., 2016). The main abnormality unearthed was a prolonged strength-duration time constant suggesting increased persistent Na^+-channels. Subsequent therapy with the non-selective Na^+-blocker, mexiletine resulted in improvement of symptoms and in the abnormalities of axonal excitability.

6.5. Metabolic neuropathy

Multiple excitability studies have been undertaken across a spectrum of neuropathies associated with metabolic disease. Initial

Please cite this article as: M. C. Kiernan, H. Bostock, S. B. Park et al., Measurement of axonal excitability: Consensus guidelines, Clinical Neurophysiology, https://doi.org/10.1016/j.clinph.2019.07.023

studies in type 1 and type 2 diabetic patients involving motor and sensory axons have provided important insights into the pathogenesis and the development of diabetic neuropathy. In type 2 diabetes, a range of abnormalities has been identified including reduction in τ_{SD}, an increase in relative refractory period, a decrease in superexcitability and subexcitability, and a "fanning-in" appearance of threshold electrotonus (Kuwabara et al., 2002b; Misawa et al., 2005a,b, 2006; Krishnan et al., 2008; Shibuta et al., 2010b; Bae et al., 2011; Sung et al., 2012; Kwai et al., 2013). These excitability findings likely reflect altered Na^+ channel function, alteration of Na^+/K^+ pump function, membrane depolarization, and increase ischaemic resistance even in the subclinical early stage of diabetes (Bae et al., 2011). In addition, altered Na^+ channel function would likely be responsible for neuropathic pain and paraesthesiae, symptoms commonly experienced by patients diagnosed with diabetic neuropathy (Misawa et al., 2009).

In terms of therapeutic intervention, abnormalities in axonal excitability responded quickly to improvement in glycaemic control. For example, Misawa et al. (2004) demonstrated that hyperglycaemia shortened the refractory period, with subsequent improvement following institution of strict glycaemic control (Kuwabara et al., 2002b; Kitano et al., 2004; Misawa et al., 2004, 2005b; Krishnan and Kiernan, 2005). Sung et al. (2017) reported that the development of axonal dysfunction in sensory axons occurred prior to and in a different fashion from that experienced by motor axons (Sung et al., 2012). Importantly, excitability studies in sensory nerves were capable of detecting dysfunction of the axonal membrane even in asymptomatic patients. In terms of longitudinal approaches, changes in superexcitability, subexcitability, τ_{SD} and threshold electrotonus become more prominent with disease progression, and eventually the compound sensory action potential decreases, reflecting axonal loss. In contrast, the changes in superexcitability, τ_{SD} and threshold electrotonus that develop in motor axons, ted to occur in the later stages of disease.

In comparison, while there have been been fewer studies in patients diagnosed with Type 1 diabetes, similar changes have been identified in axonal excitability (Arnold et al., 2013a; Kwai et al., 2016a,b) with an increase to ischaemic resistance (Weigl et al., 1989; Strupp et al., 1990). In addition, studies from Kwai and colleagues have also shown marked improvement in axonal excitability parameters in patients treated with continuous insulin therapy compared to other regimens (Kwai et al., 2015) and that glycaemic variability in Type 1 diabetes is positively correlated with axonal dysfunction (Kwai et al., 2016a). Excitability studies have also played a key role in demonstrating the close relationship between peripheral nerve function and morphology (assessed using nerve ultrasound) in type 1 diabetes, a feature that is not present in type 2 diabetes possibly due to differences in pathophysiology (Borire et al., 2018).

Excitability studies in patients diagnosed with chronic kidney disease have established significant changes in both motor and sensory excitability profiles, with a decrease in τ_{SD}, a "fanned-in" appearance of threshold electrotonus, an increase in refractoriness and a decrease in both superexcitability and subexcitability (Fig. 5B). These findings are consistent with axonal depolarization, most likely driven by hyperkalaemia prior to dialysis (Kiernan et al., 2002b; Krishnan et al., 2005b, 2006a,b,c; Arnold et al., 2013b; Krishnan et al., 2006d). A recent randomised controlled trial demonstrated that dietary potassium restriction is neuroprotective in this patient group (Arnold et al., 2017) and that a composite excitability score of superexcitability with measures of hyperpolarizing threshold electrotonus was also closer to normal values in patients randomised to the intervention arm. In addition, axonal excitability techniques have provided insight into the differential effects of various haemodialysis regimens on axonal function (Arnold et al., 2013b, 2016), as well as potential neurotoxic effects of various immune suppressants following renal transplant (Arnold et al., 2013c), allowing for the appropriate selection of treatment strategies involved in renal replacement. Similar changes have been identified in critical illness polyneuropathy, also related to extracellular potassium levels and other factors (Z'Graggen et al., 2006).

6.6. Neurotoxicity and trauma

Chemotherapy-induced peripheral neuropathy (CIPN) is a common side-effect of many cancer treatments, producing long-term nerve damage that significantly detracts from quality of life in cancer survivors. Chemotherapy typically produces sensory or sensorimotor axonal neuropathy, although the pathophysiological mechanisms are not well defined. Axonal excitability studies have been utilised across a number of chemotherapy classes to provide information regarding the mechanisms and time course of axonal damage, with the largest body of work focusing on oxaliplatin, typically used to treat colorectal cancer (Krishnan et al., 2006; Park et al., 2009a,b, 2011a,b; Heide et al., 2018), in addition to series in paclitaxel (Park et al., 2011c) and patients exposed to bortezomib (Nasu et al., 2014). In relation to studies undertaken during therapy with oxaliplatin, acute changes in axonal excitability became evident shortly after infusion and could be explained on the basis of alterations in axonal membrane Na^+ channel function, marked particularly by changes in refractoriness. Importantly, patients who demonstrated excitability abnormalities in early treatment were subsequently more likely to develop moderate to severe neurotoxicity. The findings suggest that the degree of acute nerve dysfunction may relate to the development of chronic neurotoxicity and that excitability could facilitate the early identification of Na^+ channel dysfunction in oxaliplatin-induced neurotoxicity. Additionally pronounced chronic changes in refractoriness, superexcitability and threshold electrotonus occurred in sensory axons progressively across treatment, occurring prior to reductions in peak amplitude, which may provide a method to identify patients at risk for neurotoxicity (Fig. 5C).

In terms of trauma related approaches, excitability studies undertaken immediately following spinal cord injury identified profound changes in membrane potential, with a "fanned-in" appearance in threshold electrotonus and reduced superexcitability during the recovery cycle, consistent with membrane depolarization (Lin et al., 2007; Boland et al., 2011). These changes in peripheral motor axonal excitability occurred during spinal shock, with subsequent further deterioration in axonal function, before partial recovery ensued, in conjunction with clinical changes.

Further excitability approaches have been designed to better chart the mechanisms of injury and recovery that may develop with nerve trauma. For instance, following a mechanical interruption of axonal continuity, conduction may persist in the distal stump prior to the onset of Wallerian degeneration. Experimental excitability studies undertaken in degenerating motor axons indicated a progressive increase in rheobase and increased peak threshold deviations during both depolarizing and hyperpolarizing electrotonus paralleled by an enhanced superexcitability of the recovery cycle (Moldovan et al., 2009). These changes, attributed to a nonselective loss ion channel function, could help for early detection of ongoing axonal degeneration after mechanical injury (Moldovan et al., 2012; Howells et al., 2018a).

Studies undertaken in patients undergoing peripheral nerve regeneration following trauma showed marked and persistent abnormalities in excitability: increased rheobase and τ_{SD}, larger than normal deviations during both depolarizing and hyperpolarizing threshold electrotonus and abnormalities during early recovery cycle (Moldovan et al., 2004a, 2016; Sawai et al., 2008; Krarup and Moldovan, 2009). It was proposed that these changes were due to

Please cite this article as: M. C. Kiernan, H. Bostock, S. B. Park et al., Measurement of axonal excitability: Consensus guidelines, Clinical Neurophysiology, https://doi.org/10.1016/j.clinph.2019.07.023

the persistent increase in number of nodes of Ranvier in regenerated nerves, combined with the increased activity-dependent Na^+ influx (Tamura et al., 2006; Nakata et al., 2008). In turn, such changes could lead to hyperactivity of the Na^+/K^+ pump resulting in membrane hyperpolarization (Moldovan et al., 2004b, 2016).

7. Summary

The majority of studies highlighted in these consensus guidelines demonstrate that the assessment of axonal excitability may serve as a useful technique to explore disease pathophysiology, in addition to providing utility as a biomarker for the evaluation of patient response to pharmacological interventions and for the identification of neurotoxicity (Burke and Kiernan, 2018). However, few of these studies were designed as prospective, blinded studies of diagnostic accuracy to enable the calculation of sensitivity and specificity at particular thresholds to distinguish individuals with disease from those without disease. As such, the use of axonal excitability techniques to evaluate the functional response of patients to medications or interventional strategies remains to be validated. At present there are a number of clinical trials in neurological disease states currently underway that, in addition to providing evidence about drug efficacy, may also test the ability of excitability to predict clinical outcome. As such, the role and utility of axonal excitability as a surrogate measure in phase III trials may serve to be more appropriately explored in a future update of these initial consensus guidelines.

Acknowledgements

MCK receives funding support from the National Health and Medical Research Council of Australia [Project grant numbers APP 1123026, APP 1154676]; SK receives research support from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and the Ministry of Health, Labor and Welfare of Japan; SBP is supported by a National Health and Medical Research Council of Australia Career Development Fellowship [APP 1148595]; SV receives funding support from the National Health and Medical Research Council of Australia [Project grant numbers APP 1141617, APP 1154676]; BJW is a New York Stem Cell Foundation - Robertson Investigator. SGW receives support from the Rehabilitation Research Service, U.S. Department of Veterans Affairs.

Declaration of Competing Interest

Hugh Bostock receives royalties from UCL for sales of QtracW software referred to in this article. The other authors have no conflicts of interest to disclose. All authors have approved the final paper.

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Please cite this article as: M. C. Kiernan, H. Bostock, S. B. Park et al., Measurement of axonal excitability: Consensus guidelines, Clinical Neurophysiology, https://doi.org/10.1016/j.clinph.2019.07.023

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Please cite this article as: M. C. Kiernan, H. Bostock, S. B. Park et al., Measurement of axonal excitability: Consensus guidelines, Clinical Neurophysiology, https://doi.org/10.1016/j.clinph.2019.07.023

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Please cite this article as: M. C. Kiernan, H. Bostock, S. B. Park et al., Measurement of axonal excitability: Consensus guidelines, Clinical Neurophysiology, https://doi.org/10.1016/j.clinph.2019.07.023