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Original research article

Nerve excitability in the rat forelimb: a technique to improve translational utility

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HIGHLIGHTS

- Nerve excitability studies provide indirect measures of ion channel function *in vivo*.
- Rat nerve excitability was assessed in ulnar, tibial and tail motor axons.
- Ulnar nerve measures were longitudinally reproducible.
- Each nerve demonstrates a distinct nerve excitability profile.
- This may have functional and pathological implications.

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ABSTRACT

Background: Nerve excitability testing by threshold-tracking is the only available method to study axonal ion channel function and membrane potential in the clinical setting. The measures are, however, indirect and the interpretation of neuropathic changes remains challenging. The same multiple measures of axonal excitability were adapted to further explore the pathophysiological changes in rodent disease models under pharmacologic and genetic manipulations. These studies are typically limited to the investigation of the "long nerves" such as the tail or the tibial nerves.
New method: We introduce a novel setup to explore the ulnar nerve excitability in rodents. We provide normative ulnar data in 11 adult female Long Evans rats under anaesthesia by comparison with tibial and caudal nerves. Additionally, these measures were repeated weekly on 3 occasions to determine the repeatability of these tests.
Results: Nerve excitability assessment of ulnar nerve proved to be a longitudinally repeatable measure of axonal function mature in rats, as were measures in tibial and caudal nerves.
Comparison with existing method: Ulnar nerve motor excitability measures were different from the caudal and tibial excitability measures. Most notably, ulnar nerve showed the largest threshold changes during both depolarizing and hyperpolarizing threshold electrotonus.
Conclusions: Ulnar nerves demonstrate a distinct nerve excitability profile than the caudal and tibial nerves which could have functional and pathological implications.

1. Introduction

Peripheral nerve injury occurs due to a wide range of aetiologies, including diabetes, toxin exposure, spinal cord injury and inflammatory conditions. Neuroprotection for peripheral nerve injury remains elusive despite promising data in animal models (Scheib and Hoke, 2013). A lack of common measurement techniques is one factor that may hinder translation from animal to human research.

Nerve excitability techniques are sophisticated electrophysiological methods that enable the indirect assessment of peripheral nerve ion channels, pumps, exchangers and membrane potential (Kiernan et al., 2000). These techniques have been utilised

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in the clinical setting on a variety of neurological disorders such as motor neuron disease, spinal cord injury, stroke and peripheral neuropathies (Winhammar et al., 2005; Krarup and Moldovan, 2009; Krishnan et al., 2009; Boland et al., 2011; Arnold et al., 2013; Huynh and Kiernan, 2015). Though these studies have provided important insights, comprehensive interpretation is often challenging in a clinical setting (Boerio et al., 2011). As such, the use of these methods in animal models of neurological disorders provides an opportunity to investigate the molecular mechanisms that underpin specific nerve excitability changes and thus advance translational research (Moldovan and Krarup, 2004; George and Bostock, 2007; Moldovan et al., 2009; Boerio et al., 2010).

Neurological disorders often demonstrate a preferential pattern of involvement. As such, it is important that functionally and pathologically relevant nerves are investigated (Wang et al., 2008). Previous studies of nerve excitability in rodent models have largely focused on caudal motor nerves (Yang et al., 2000; Schwarz et al., 2006; George and Bostock, 2007), some other studies have utilised the tibial nerve (Susuki et al., 2007; Moldovan et al., 2009, 2012; Rosberg et al., 2013), though none have investigated any forelimb nerves. Importantly, nerve excitability tests in the clinical research are almost exclusively conducted on the upper limb making extrapolation from rodent to human neuropathies ambiguous. Furthermore, investigation of forelimb nerves provides a functionally advantageous scenario whereby measures of grip strength, dexterity and clinically relevant injuries may also be measured (Wang et al., 2008).

The purpose of this study was to develop a nerve excitability protocol in the rat forelimb, specifically in the ulnar nerve, and compare it with previously established protocols in caudal and tibial motor nerves. Additionally, these measures were repeated weekly on 3 occasions to determine the repeatability of these tests. Therefore, this study aimed to examine the nerve excitability profiles of 3 physiologically distinct nerves and assess the longitudinal utility of these measures in healthy mature rats.

2. Methods

2.1. Animals and experimental design

This study was approved by the Danish National Animal Experimental Committee in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. Studies were undertaken using 11 adult female Long Evans rats (Taconic, France) weighing 180 g (corresponding to 8 weeks of age). The rats were housed in pairs in an animal facility with 12-h dark light cycle with rat chow and water ad libitum. Following a one-week acclimatisation period (i.e. ~9 weeks old; weight 200 ± 4 g) assessments were commenced and longitudinal assessments were conducted each week for 2 weeks (~10 weeks old; weight 210 ± 3 g and ~11 weeks old; 218 ± 4 g).

The rats were placed under general anaesthesia using a 1:1 mixture of Hypnorm/Midazolam (5 mg/ml) injected subcutaneously, with an induction dose of 0.2 ml per 100 g followed by a maintenance dose of 50% every 30 min there-after for up to 1.5 h. Body temperature was measured and maintained on a temperature controlled pad (HB 101/2, LSI Letica) set to 37 °C. Longitudinal studies were conducted to assess repeatability, with assessments conducted 3 times approximately 1week apart (n = 11).

2.2. Electrophysiological setup

Electrophysiological studies were undertaken using a constant current stimulator (DS4, Digitimer Ltd.). The amplified signal (10Hz–6 Hz, Neurolog system) was digitized by a computer with an analog-to-digital board (National Instruments) at a sampling rate of 10 Hz. Compound motor action potential (CMAP) amplitude, measured peak-to-peak, CMAP latency, measured to half-peak, and nerve excitability parameters were obtained from all three nerves during each assessment.

Peripheral nerve excitability set-up: The limb or tail was placed on a piece of hydrophobic cotton to reduce the stimulus artefact and fixed (but not stretched) with a clamp. Stimulus was delivered via custom-made platinum needle electrodes. Compound muscle action potentials were recorded subcutaneously using needle electrodes. Electrophysiological tests were undertaken to compare hindlimb (tibial), forelimb (ulnar), and tail (caudal) motor nerves (Fig. 1) using the following stimulation and recording setups: 1) Hindlimb – the tibial nerve: stimulation via a cathode at the ankle and anode at the base of the tail, recording over the plantar muscles of the foot with the reference placed in the 3rd digit (Fig. 1A). 2) Forelimb – the ulnar nerve: stimulation via a cathode at the elbow and anode 1 cm proximally in the axillar region, recording over hypothenar muscles with the reference electrode placed at the 4th digit (Fig. 1B). 3) Tail –the caudal motor nerve: stimulated via a cathode at the base of the tail and anode at the thigh 2 cm away, recording electrodes placed 4.5 cm distally with the reference electrode placed a further 1 cm distally (Fig. 1C). Full nerve excitability recordings from all 3 nerves was completed in approximately 1 h and the sequence of nerves tested was randomised to minimise the influence of anaesthesia time on nerve excitability (Osaki et al., 2015).

2.3. Nerve excitability testing

Nerve excitability parameters were recorded using TRONDNF protocol with QTRACS software (© Institute of Neurology, London, United Kingdom). This protocol included five testing paradigms namely stimulus response behaviour, strength duration relationship, threshold electrotonus (TE), current-threshold (I/V) and the recovery cycle which are discussed in detail elsewhere (Kiernan et al., 2000; Boerio et al., 2009). Briefly, a 1 ms stimulus is manually increased at 0.2 mA increments until a supramaximal compound action potential is reached, the computer then progressively decreases the stimuli to generate a stimulus response curve. The software then 'tracks' the stimulus required to generate a target response (40% of maximal compound action potential) as the quantitative measure for the remainder of the protocol. Changes to the target response are induced using a range of conditioning stimuli. The strength duration time constant (SDTC) was calculated from change in stimulus required to elicit the target response to four different stimulus widths: 0.2 ms, 0.4 ms, 0.8 ms and 1 ms (Bostock, 1983; Mogyoros et al., 1996). The slope of the linear relationship derived from SDTC provides the value for rheobase. Threshold Electrotonus (TE) was assessed using 100 ms depolarising and hyperpolarising currents at ±40%, ±20% delivered along with long hyperpolarising polarising currents of 150 ms at −70%, and 200 ms at −100% of the control threshold. Changes induced by these polarising currents were assessed at multiple time points during and after the stimuli (Bostock et al., 1998; Kiernan et al., 2000). Current-threshold (I/V) relationship was measured at a single time point following 200 ms polarising currents ramped from +50% to −100% of the control threshold. The recovery cycle assessed the restoration of axonal excitability following a supramaximal stimulus at multiple time points from 2 ms to 200 ms, this measure provides an indirect measure of nodal and paranodal Na^+ and K^+ conductances via four main parameters: refractoriness, relative refractory period, superexcitability and subexcitability (Kiernan et al., 2000).