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Excitability of sensory axons in amyotrophic lateral sclerosis

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HIGHLIGHTS

- Excitability properties of sensory axons were investigated in ALS patients.
- Axonal membrane properties were normal in sensory nerves of ALS.
- Sensory dysfunction may result from a ganglionopathy or more proximal central abnormalities.

ABSTRACT

Objective: To evaluate the excitability of sensory axons in patients with amyotrophic lateral sclerosis (ALS).
Methods: Comprehensive sensory nerve excitability studies were prospectively performed on 28 sporadic ALS patients, compared to age-matched controls. Sensory nerve action potentials were recorded from digit 2 following median nerve stimulation at the wrist. Disease severity was measured using motor unit number estimation (MUNE), the revised ALS Functional Rating Scale (ALSFRS-R) and the MRC scale.
Results: There were no significant differences in standard and extended measures of nerve excitability between ALS patients and controls. These unchanged excitability measures included accommodation to long-lasting hyperpolarization and the threshold changes after two supramaximal stimuli during the recovery cycle. Excitability parameters did not correlate with MUNE, ALSFRS-R, APB MRC scale or disease duration.
Conclusions: This cross-sectional study has identified normal axonal membrane properties in myelinated sensory axons of ALS patients. Previously described sensory abnormalities could be the result of axonal fallout, possibly due to a ganglionopathy, or to involvement of central sensory pathways rostral to gracile and cuneate nuclei.
Significance: These results demonstrate the absence of generalized dysfunction of the membrane properties of sensory axons in ALS in the face of substantial deficits in motor function.

1. Introduction

Cumulative evidence suggests that some ALS patients have dysfunction in non-motor systems, consistent with the concept of a multisystem disorder that extends beyond the classic pathological view (Tanaka et al., 1984; Heads et al., 1991; Ince et al., 1996; Kiernan et al., 2011; Turner and Swash, 2015; Al-Chalabi et al., 2016; McCombe et al., 2017). These non-motor abnormalities include cognitive and behavioural change, extrapyramidal and cerebellar dysfunction, amongst others (Turner et al., 2013). While these abnormalities do not occur in all ALS patients, they add a new layer of complexity to the disease process. In addition, these features affect disease progression and prognosis. Cogent evidence for abnormalities in peripheral and central sensory pathways in ALS patients has come from clinical neurological examination (Pugdahl et al., 2007), electrophysiological studies (Radtke et al., 1986; Gregory et al., 1993; Mondelli et al., 1993; Isaacs et al., 2006; Iglesias et al., 2015; Isak et al., 2016b) and pathological studies (Heads et al., 1991; Isaacs et al., 2006; Dalla Bella et al., 2016). Specifically, tissue investigations have shown structural changes in sensory axons of ALS patients including evidence of axonal degeneration and reinnervation, preferentially affecting large-calibre

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myelinated fibres (Heads et al., 1991; Hammad et al., 2007). However, the precise pathophysiological mechanisms involved in this process remain unknown.

Nerve excitability studies using threshold-tracking techniques are an established non-invasive approach that can detect changes in the properties of the axonal membrane, including the function of ion channels, pumps and transporters, of myelinated axons *in vivo* (Bostock et al., 1998; Krarup and Moldovan, 2009; Kiernan and Kaji, 2013), complementing information obtained using conventional nerve conduction studies (Burke et al., 2001). Excitability studies have reported abnormalities of the membrane properties of motor axons in ALS patients, characterized by prolonged strength-duration time constant, increased superexcitability, and changes in threshold electrotonus (Mogyoros et al., 1998b; Kanai et al., 2006; Vucic and Kiernan, 2006). Such abnormalities reflect increased persistent Na^+ and decreased K^+ conductances, consistent with hyperexcitability of the axonal membrane, and likely contribute to the clinical features of ALS such as fasciculation and cramp (Vucic et al., 2013; Simon et al., 2014; de Carvalho et al., 2017). Regarding axonal function in sensory axons, an initial study showed normal strength-duration time constant, refractoriness at 2 ms and superexcitability at 7 ms in ALS patients (Mogyoros et al., 1998b). However, a parallel study compared the changes in axonal excitability produced by ischaemia, and reported greater post-ischaemic hyperpolarization in median nerve sensory axons of patients with ALS (Mogyoros et al., 1998a), a finding that might suggest an abnormality of inward rectification due to the hyperpolarization-activated current, I_h.

Nerve excitability studies have since been extended, and currently available protocols can better define the activity of I_h (Tomlinson et al., 2010; Howells et al., 2012) and of K^+ currents (Shibuta et al., 2010). The aim of this study was to explore the membrane properties of sensory axons in patients with ALS using extended nerve excitability protocols to shed light on any pathophysiological mechanisms that may be responsible for sensory symptoms in ALS.

2. Materials and methods

Patients were prospectively recruited from the ForeFront Clinic, Brain and Mind Centre, as part of the NHMRC Sydney Health Partners collaboration at the University of Sydney, Australia. In total, 48 subjects were studied, 28 patients with sporadic ALS and 20 age-matched healthy controls. All participants provided written informed consent in accordance with the Declaration of Helsinki. The research was approved by the Human Research Ethics Committee of the University of Sydney.

Patients were classified as probable or definite according to the Awaji criteria (de Carvalho et al., 2008), and were clinically staged using the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) (Cedarbaum et al., 1999) and MRC rating scale (Medical Research Council, 1976). The rate of change for disease progression (ΔALSFRS-R) was also calculated as previously reported (Labra et al., 2016). Disease duration was defined as the time in months from the onset of symptoms to the date of study. A complete neurological examination and diagnostic electromyography and nerve conduction study following standard techniques (Kimura, 2001) were performed by a neurologist (JM or TD). Specifically, on the sensory neurological examination, vibration, light touch, position sense, pinprick and cold/heat sensation were assessed in proximal and distal areas of the limbs. Subjects with a history of carpal tunnel syndrome, polyneuropathy or diseases or drugs that could produce polyneuropathy were excluded. Axonal excitability studies were compared with an age-matched cohort of 20 healthy controls, who were not taking medications with known neurological effects.

2.1. Axonal excitability assessment

The excitability of sensory axons in the median nerve was studied by threshold tracking using the TRONDNF protocol (© Institute of Neurology, Queen Square, UCL, London, UK; Bostock et al., 1998; Kiernan et al., 2000, 2001a). Excitability studies were undertaken on the side with more severe motor dysfunction, regardless of the level of impairment. Briefly, the antidromic sensory nerve action potentials (SNAPs) were recorded from digit 2 using disposable Ag/AgCl self-adhesive ring electrodes (RE-D; Electrode Store, Enumclaw, WA, USA), with two ring electrodes approximately 4 cm apart, with the proximal electrode around the proximal phalanx and the distal electrode around the middle phalanx. The stimulation of the median nerve at the wrist was performed using non-polarizable Ag/AgCl adhesive electrodes (White Sensor 4500 M-H; Ambu, Ballerup, Denmark). The cathode was placed over the median nerve and the anode 10 cm proximal, on the radial edge of the forearm. Electrical stimulation was delivered by a linear bipolar constant-current source (DS5; Digitimer; Welwyn Garden City, UK). The amplified signals had mains frequency contamination removed in-line using a HumBug 50/60 Hz Noise Eliminator (Quest Scientific; North Vancouver, BC, Canada), and were then digitised with a 16-bit data acquisition system (NI-USB6251; National Instruments; Austin, TX, USA). The stimulation protocol and data acquisition were under computer control using threshold tracking software (QtracS; © Prof Hugh Bostock, Institute of Neurology, UCL, UK). Skin temperature was monitored at the site of stimulation during the excitability recordings to ensure a temperature of approximately 32 °C (Burke et al., 1999; Kiernan et al., 2001a). The wrist and forearm were wrapped in a warm towel, and studies commenced only when the temperature was stable and greater than 32 °C.

The excitability protocol includes five parts: (i) stimulus-response relationship (SR), (ii) strength-duration properties (QT), (iii) threshold electrotonus (TE), (iv) current-threshold relationship (I-V), and (v) recovery cycle (RC). The stimulus-response relationship measures the response to graded 0.5 ms-wide rectangular constant current pulses (test stimuli) and was used to determine the stimulus-response properties and the size of the target potential (40% of the maximal SNAP). The strength-duration properties quantify the increase in stimulus current required to produce a target response as the duration of the stimulus decreases (0.5, 0.4, 0.3, 0.2 and 0.1 ms). The rheobase and strength-duration time constant were defined by linear regression of stimulus charge against stimulus duration using Weiss' law (Mogyoros et al., 1996). Threshold electrotonus examines the changes in excitability before, during and after long lasting subthreshold conditioning currents. The standard protocol measures changes in response to 100-ms long depolarizing and hyperpolarizing currents of ±40% and ±20% strength of the threshold stimulus (control) for the unconditioned test potential. To explore inward rectification better, the protocol was extended to include a 200-ms hyperpolarizing current, the strength of which was 70% of the control threshold, and a 300-ms hyperpolarizing current at 100% of the control threshold (Tomlinson et al., 2010; Howells et al., 2012). The current-threshold relationship quantifies the rectifying conductances (inward and outward) and the resting input conductance. The threshold of the test potential was measured at the end of a 200-ms conditioning current, the strength of which was changed in 10% steps from +50% of the threshold in the depolarizing direction to −100% in the hyperpolarizing direction. The recovery cycle assesses changes in excitability following a supramaximal conditioning stimulus. Initially axons are inexcitable (absolute

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