Clinical Neurophysiology Practice 7 (2022) 319–324

Contents lists available at ScienceDirect

Clinical Neurophysiology Practice

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

Research paper

The threshold tracking nerve conduction study technique: Experience of clinical users unfamiliar with a research-grade neuronal excitability system

C.E. McIlduff [a,*], B.J. Wainger [b,c], R.L. Freeman [d], S. Samaan [a], I. Yator [a], H. Gutierrez [a], S. Verga [a], S.B. Rutkove [a]

[a] Department of Neurology, Beth Israel Deaconess Medical Center, Boston, MA, USA
[b] Department of Neurology, Sean M. Healey & AMG Center for ALS, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[c] Department of Anesthesiology, Critical Care and Pain Medicine, Massachusetts General Hospital, Boston, MA, USA
[d] Center for Autonomic and Peripheral Nerve Disorders, Beth Israel Deaconess Medical Center, Boston, MA, USA

ARTICLE INFO

Article history:
Received 20 April 2022
Received in revised form 4 August 2022
Accepted 22 August 2022
Available online 13 October 2022

Keywords:
Axonal excitability
Biomarker
Clinical electrophysiology
Neuromuscular disorders
Threshold tracking
Nerve conduction studies

ABSTRACT

Objective: To 1) explore if clinical electrophysiologists with different degrees of experience performing standard nerve conduction studies could run a threshold tracking nerve conduction study (TTNCS) protocol and 2) learn how clinical users view a research-grade TTNCSs neuronal excitability system.
Methods: Five clinical electrophysiologists conducted a TTNCS session using QTracS and then completed a questionnaire describing their impressions.
Results: All of the electrophysiologists completed the QTracS protocol on an initial attempt. Perceived strengths comprised the ease of preparatory steps and quick protocol speed. Identified drawbacks included an unwieldly user-interface. The electrophysiologists indicated that knowledge of TTNCS principles and applications would be critical for incorporation of the method into clinical use.
Conclusions: This pilot study suggests that clinical electrophysiologists can carry out TTNCSs with a research-grade system. The development of a more user-friendly program, along with dedicated education and training, could lead to wider application of the TTNCS technique.
Significance: Considered together with clinical presentation and other biomarkers, increased use of TTNCSs could provide improved assessment of neuromuscular disease and treatment response.

1. Introduction

There is a need for objective measures of neuronal function to characterize the pathophysiology of neuromuscular disorders and guide the development and choice of therapies for debilitating symptoms (Diester et al., 2016; Smith et al., 2017; Tracey et al., 2019). Symptoms can be negative (e.g., numbness) and positive (e.g., pain and tingling), and can reflect decreases and increases in baseline nerve excitability respectively. Some of the physiological correlates of these symptoms, which are related to alterations in axonal membrane properties and ion channel and pump function, can be objectively assessed by a powerful approach called neuronal electrical excitability testing (Bostock et al., 1998; Krishnan et al., 2008). Neuronal excitability testing can be performed using a research-grade technique pioneered by Hugh Bostock, PhD, FRS of University College London (Kiernan et al., 2020). This quantitative approach employs a specialized form of NCSs called threshold tracking nerve conduction studies (TTNCSs).

TTNCSs are designed to gauge the excitability of axons (Bostock et al., 1998; Kiernan et al., 2020). The fundamental goal of the approach is to monitor the amount of stimulation, (i.e., the amount of electrical charge in a stimulus of prescribed intensity and duration), it takes to generate an action potential with an amplitude of pre-determined submaximal magnitude; this target response size is defined as the 'threshold'(Bostock et al., 1998; Kiernan et al., 2020). Stimulus intensity is increased or decreased so that response amplitudes match the threshold as consistently and closely as possible while various types of conditioning stimuli are

* Corresponding author at: Department of Neurology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, TCC 810, Boston, MA 02215, USA.
E-mail addresses: cmcilduf@bidmc.harvard.edu (C.E. McIlduff), brian.wainger@mgh.harvard.edu (B.J. Wainger), rfreeman@bidmc.harvard.edu (R.L. Freeman), ssamaan@bidmc.harvard.edu (S. Samaan), iyator@bidmc.harvard.edu (I. Yator), hgutier1@bidmc.harvard.edu (H. Gutierrez), sverga@bidmc.harvard.edu (S. Verga), srutkove@bidmc.harvard.edu (S.B. Rutkove).

https://doi.org/10.1016/j.cnp.2022.08.003
2467-981X/

applied (Bostock et al., 1998; Kiernan et al., 2020). Furthermore, different stimulation patterns can be employed to alter membrane potential and evaluate how ion channel sub-types (e.g., persistent and transient sodium, slow and fast potassium, and hyperpolarization activated cation conductance channels) function in health and disease (Kiernan et al., 2020). By providing unbiased biomarker information, the TTNCS technique has potential for broader application – including in the realms of both clinical and research neurophysiology.

In research trials, TTNCSs have successfully been used to investigate sensorimotor conditions ranging from motor neuron diseases to various polyneuropathies (Bostock et al., 1998; Emery et al., 2011; Kiernan et al., 2020; Krishnan et al., 2008; Tomlinson et al., 2010). Further, the method has been combined with other modalities, such as microneurography, to evaluate small fibers that play a role in autonomic function and pain (Burakgazi et al., 2011). As a non-invasive approach that implements similar principles, TTNCSs are an extension of standard NCSs; however, TTNCSs yield more detailed, sophisticated data (Fig. 1).

Despite its clear value, TTNCSs have not been incorporated into routine clinical care (Bostock et al., 1998; Kiernan et al., 2000). In the past, factors including the absence of commercially-available devices and the time required to do the procedure were proposed as barriers to more widespread use (Bostock et al., 1998; Kiernan et al., 2000). Yet practice has not markedly changed since the development of TTNCS equipment and protocols that can be used to measure sensorimotor nerve excitability within about 20 min (Kiernan et al., 2000; Kiernan et al., 2001). Further, two recent multicenter clinical therapeutic trials incorporated TTNCSs after a single in-person training session, suggesting that experienced neurophysiologists can quickly learn and accurately apply the method (Wainger et al., 2021; Weiss et al., 2021).

We therefore surmised that other reasons account for why the technique has not been more readily adopted. The notion that one needs extensive experience performing NCSs as a prerequisite to conduct TTNCSs and an abstruse user-interface were considered potential explanations. Thus, the goals of this pilot study were to 1) explore if a small group of electrophysiologists with different degrees of experience performing standard nerve conduction studies could run a threshold tracking nerve conduction study (TTNCS) protocol and 2) learn how clinical users view a research-grade TTNCSs neuronal excitability system.

2. Methods

The Beth Israel Deaconess Medical Center Institutional Review Board approved the study protocol. All subjects provided written consent prior to participation. All procedures were performed in accord with the Helsinki Declaration of 1975.

2.1. Participants

Two healthy volunteers were recruited to undergo standard and threshold tracking NCSs. Inclusion criteria for the volunteers included a normal neurological examination and a normal median sensory nerve action potential (SNAP) when performed using standard NCS technique. A total of five clinicians and EMG technologists with experience performing standard NCSs but with no prior experience performing TTNCSs were recruited to perform a TTNCS session. They were considered 'naïve' users.

2.2. Electrophysiology equipment

TTNCSs were performed using the QTracS software (© UCL Institute of Neurology, London, UK, available from Digitimer Ltd at Digitimer,com), a DS5 isolated bipolar stimulator (Digitimer Ltd, Sussex, UK), and a National Instruments USB-6341 (Part 782251–01) card for analog-to-digital conversion (Fig. 2) (Kiernan et al., 2020). The DS440-2 isolated EMG amplifier (Dig-

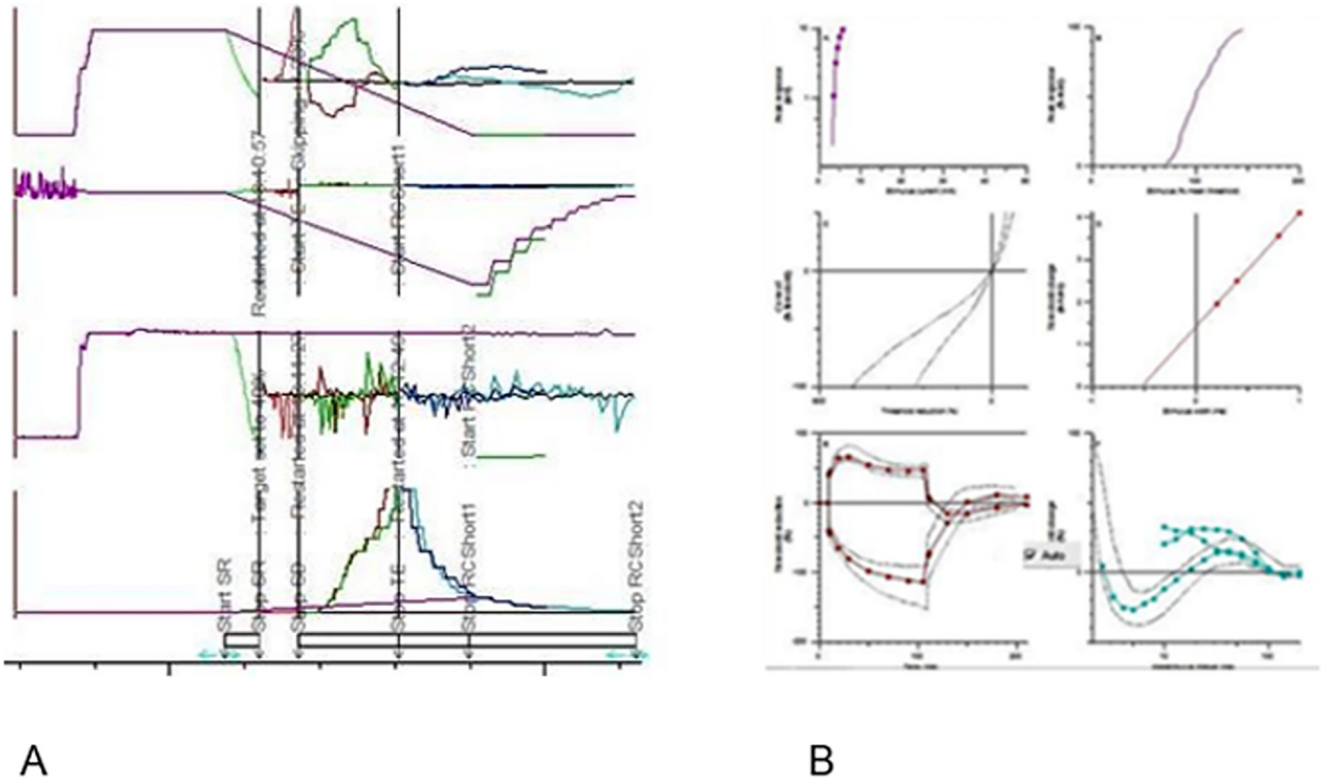

Fig. 1. A) and B). The data generated by TTNCSs.

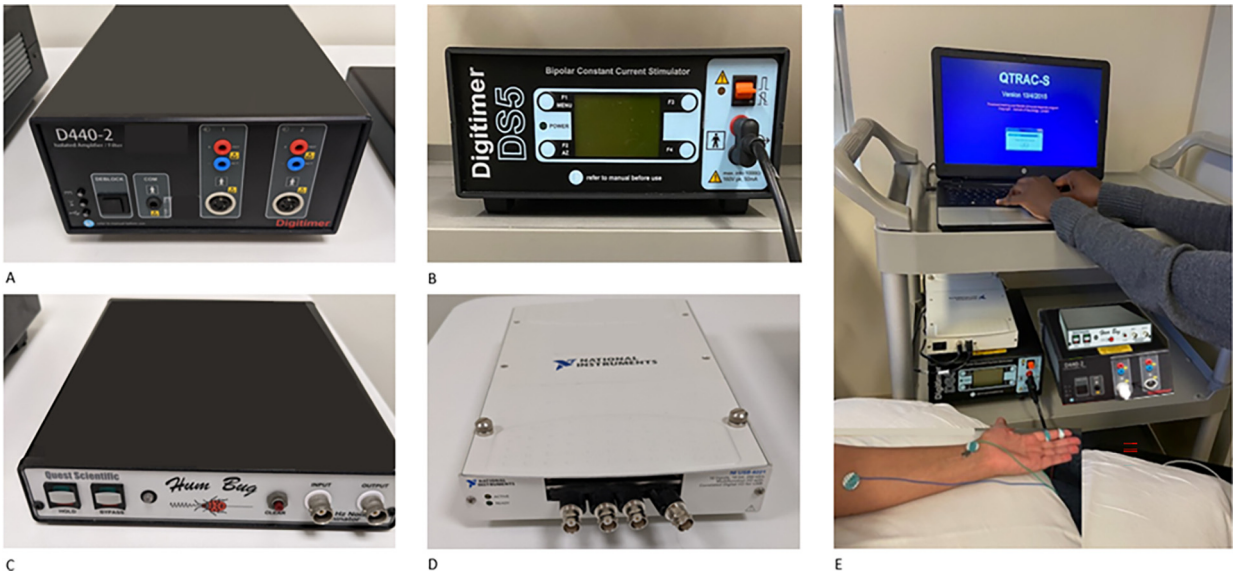

Fig. 2. Electrophysiology equipment for TTNCSs: A) D440-2 isolated EMG amplifier B) DS5 isolated bipolar stimulator C) Humbug noise eliminator D) National Instruments USB-6341 card for analog-to-digital conversion E) Complete system setup including laptop used to run the threshold tracking software and to alter stimulation intensities.

itimer Ltd, Sussex, UK) and Humbug noise eliminator (Quest Scientific, Vancouver, Canada) component were also employed. A laptop was used to run the threshold tracking software and to alter stimulation intensities. Responses were recorded using non-polarized ring electrodes. A thermometer was used to ensure a skin temperature between 31 and 33 °C on the tested limb.

2.3. Instruction manual

Building on the manual prepared for prior clinical trials, a TTNCS instruction manual was designed (Supplementary File) (Wainger et al., 2021; Weiss et al., 2021). An introduction with details regarding participant positioning for the procedure, electrode placement, and electrode connections to the DS5 were provided along with pictures. Starting with directions to click on the appropriate icon on the laptop, electrophysiologists were then guided through a 38-page step-by-step presentation with screenshots of expected prompts throughout the QTracS protocol. No background information on the principles or uses of TTNCSs was provided to the naïve users. Therefore, they did not have a detailed conceptual understanding of the technique. We purposely avoided providing TTNCS educational material and training in order to focus on user experience with the research-grade system itself.

2.4. Standard and threshold tracking nerve conduction studies

Standard sensory NCSs of the right median nerve recording from digit 2 were performed to ensure a normal response. Then TTNCSs were performed using the QTracS protocol (Kiernan et al., 2020). Stimulus response, strength-duration time constant, threshold electrotonus, current-threshold (I/V) relationship, and recovery cycle data were obtained when stimulating the right median sensory nerve and recording from digit 2 (Kiernan et al., 2020).

2.5. Outcome measures

The time required to complete a session was recorded. The clinical electrophysiologists completed a 9-item forced-choice and open-ended questionnaire describing their experience with the protocol. The survey covered topics including the logistical aspects of the interface, user grasp of the TTNCS protocol, any challenges encountered while running the session, and perceived drawbacks and strengths of the research-grade neuronal excitability system (Fig. 3). Following TTNCSs, the healthy volunteers on whom testing was performed rated pain on a scale of 0–10, with 0 being no discomfort and 10 being the most discomfort.

3. Results

3.1. Participants

The electrophysiology group comprised five different individuals with 9 months to 8 years of experience performing traditional NCSs, including an EMG technologist, 2 clinical electrophysiology/neuromuscular medicine fellows, and 2 attending clinical neurophysiologists. None of the electrophysiologists had performed the TTNCS technique in any formal capacity. One of the electrophysiologists was familiar with the basic principles of TTNCSs. Two healthy women (aged 28 and 33 years) underwent TTNCSs for all sessions. Each had a normal neurological examination and a normal median sensory nerve action potential on standard NCSs.

3.2. Feasibility

All naïve users were able to successfully run a full QTracS protocol on an initial attempt. Each study took an average of 34 (range 31–41) minutes. Naïve user data plots appeared as expected for a healthy individual in three of five cases. With the exception of a single rating in the range of 3–7 during the recovery cycle of one session, healthy participants consistently rated discomfort as minimal (i.e., between 0 and 2).

3.3. Clinician Feedback on usability of research-grade neuronal excitabilty system

One of the clinical electrophysiologists felt that the current research-grade system left little room for user error beyond electrode placement, a true strength. Further, they indicated that it was easy to set up and was faster to perform than they had expected. However, all users thought that key logistical steps

User Experience Questionnaire

User ID Number:
Date:
Total Time of Session:

1. Could you tell when the waveform was supramaximal?	□ Yes □ No
2. Do you know which wave form you should be looking at?	□ Yes □ No
3. Did you understand why you had to turn the DS stimulator button on and off?	□ Yes □ No
4. Did you encounter any difficulties in running the program? If so what?	□ Yes □ No
5. Did you understand what each part of the protocol was assessing? • Strength duration (SD) • Threshold electrotonus (TE) • Threshold I/V (IV) • Recovery Cycle (RC)	□ Yes □ No □ Somewhat
6. Did you understand why you were completing legends and the dialogue box?	□ Yes □ No
7. What did you like about the program?	
8. What did you dislike about the program?	
9. Other comments if any:	

Fig. 3. Clinical User Survey.

required to navigate the program (e.g., button-pushing, information entry) lacked sufficient clarity. Additionally, only one of the five users could tell which segment of the axon excitability protocol the semi-automated TTNCS program was testing throughout the session. The majority (80 %) of clinicians felt they encountered some 'obstacle' when running the program. For instance, they had trouble identifying the SNAP and other items on the display. In other cases, the clinical users could not discern what the TTNCS program was evaluating or why they were being asked to perform certain tasks to advance the program. Finally, the users felt that available references for expected outcomes were deficient. Some of these themes overlapped with perceived drawbacks, which included the convoluted nature of the user interface. Distinct from the interface and protocol, users also highlighted how a lack of understanding of the TTNCS program and related data was a limitation; without a better grasp of the stimulation protocol, a respondent volunteered, it would be difficult to counsel patients on what to anticipate (Table 1).

4. Discussion

There is a need for objective measures of nerve health for use in therapeutic trials and clinical care (Diester et al., 2016; Smith et al., 2017; Tracey et al., 2019). While valuable, existing approaches to the diagnosis and monitoring of neuromuscular disorders have disadvantages including subjectivity, cost, limited localizing value, and an inability to address associated pathophysiology (Colloca, 2019; Koulouris et al., 2020; Lauria et al., 2010). As a technique that quantifies axon excitability and can provide advanced information about the physiological features and mechanisms of neuronal dysfunction, TTNCSs seem well-suited to serve as a valuable extension of traditional NCSs (Kiernan et al., 2020). However, use of the method has not been as common as one might expect based on its potential value, and has generally not extended beyond a research environment to more standard clinical use (Bostock et al., 1998; Kiernan et al., 2000).

This preliminary work demonstrated how electrophysiologists with varying degrees of expertise performing standard NCSs could run a TTNCSs protocol - even without formal training. In fact, user inexperience with TTNCS had the potential to negatively influence the physical and psychological experience of healthy participants throughout the test session. Nonetheless, the procedure was extremely well-tolerated.

Another perceived benefit of the semi-automated QTracS protocol, according to the clinical electrophysiologists who participated in this study, was a limited chance for user error beyond the placement of electrodes. If this were true, TTNCSs could have some advantage over standard NCSs which can have high examiner and test–retest variability (Chaudhry et al., 1991; Chaudhry et al., 1994; Dyck et al., 1991; Salerno et al., 1999). Importantly, this user's observation was based on experience running a single QTracS session, and does not necessarily apply to the entire TTNCS data collection and analysis process. As with any neurophysiological technique, there are important potential pitfalls inherent to the performance and analysis of TTNCSs, including the effects of noise, temperature, changes in baseline threshold, and medication use (Kiernan et al., 2020). Although clinical electrophysiologists with experience performing standard NCSs have transferrable skills, TTNCSs require training to trouble-shoot and reliably perform high-quality recordings in an efficient manner.

In fact, while naïve users consistently completed the QTracS session, it did take them about 1.5 times as long as 'expert' users

Table 1
Clinician Feedback on the Usability of a Research-Grade Neuronal Excitabilty System.

Questionnaire Item	Percent of clinical users
User Interface: thought key technical elements, logistical steps were clear (i.e., could identify sensory nerve action potential (SNAP) on screen, could determine when SNAP was supramaximal, understood why to turn DS5 stimulator button on/off, understood why to enter information into legends and dialog boxes)	0
TTNCS Protocol: completely understood what each part of the threshold tracking nerve condction study (TTNCS) protocol (i.e., strength duration time constant, threshold electrotonus, current-threshold (I/V) relationship, recovery cycle) was assessing	20
Obstacles: encountered some difficulty while running program • Could not see SNAP or determine if supramaximal stimulation was achieved • Had trouble locating items on display • Did not understand program, what evaluating, why performing each step • Did not know what normal values or expected outcomes were	80
Perceived Drawbacks: • User interface unintuitive • Display had so much information, limited labeling that it was difficult to focus attention • Difficult to identify what parts of TTNCS protocol were being evaluated and when • Small font • Did not understand program, why performing each step • Did not know what normal values or anticipated outcomes were • Did not know how to use data generated • Without knowledge of stimulation protocol, no way to explain experience and expectations to patients	
Perceived Strengths: • Easier set-up and overall use than expected • Faster than expected • Little room for user error beyond clicking buttons and initial electrode placement	

to complete the protocol as reported in earlier work (Kiernan et al., 2000; Kiernan et al., 2001). The extended time requirement could have been related to many factors, including a lack of practice with an unfamiliar procedure and a complicated user interface. Indeed, this pilot study suggests that new clinical users found it challenging to navigate the logistical steps of the current research-grade TTNCS system. Since we purposefully recruited individuals who had no formal experience performing TTNCSs, it is not surprising that only the individual with some background familiarity with principles of TTNCS would comprehend the goals of individual stimulation sequences in QTracS. Nonetheless, the finding that 80 % of naïve clinical users had trouble identifying important components of the protocol (e.g., the SNAP on the screen, the title of each stimulation sequence) highlights how the current user-interface is not straightforward. While the technology required to match the complexity and breadth of TTNCSs could help explain this factor, clearer labeling and simplified displays could be conducive to clinical implementation.

However, as highlighted by some of the survey responses, even the most user-friendly software would be no substitution for education on the principles and applications of TTNCSs. While we purposefully recruited individuals who lacked experience with TTNCSs to perform the procedure, this approach does not mirror real-world research or clinical practice. Just as standard NCSs are an extension of the clinical presentation so, too, are TTNCSs (Kiernan et al., 2020). Education would facilitate a better grasp of how TTNCSs can complement existing forms of evaluation, how to implement different axon excitability tests, allow trouble-shooting and data integrity checks, and provide insight into how to interpret the data generated. No single TTNCS result can be used to diagnose a specific disorder. Rather, axon excitability profiles derived from a panel of TTNCS assessments can be considered together with other clinical information to gain insight into the pathophysiology of disease (Kiernan et al., 2020). Training is critical, therefore, to understanding how to effectively implement and interpret TTNCSs.

To our knowledge, TTNCS principles and techniques are not standard elements in residency, fellowship, or continuing medical education curricula. For this reason, a failure to more routinely apply the technique could be partly due to a lack of awareness about the method. While exploring this possibility was beyond the scope of this pilot project, it remains a topic for future research. Similarly, further trials could investigate the perceived value of TTNCSs from the perspectives of research and clinical electrophysiologists and characterize how TTNCSs influence approaches to care.

The reproducibility and within- and between-subject variability of TTNCSs have previously been evaluated (Tomlinson et al., 2010). It is worth noting that we did not comprehensively assess these parameters or the quality of recordings in our study. Another limitation of this study was the small number of participants. As a result, the findings are not necessarily generalizable, including across different populations and settings.

5. Conclusions

Considered together with other neurophysiological measurements and study biomarkers, TTNCSs data could be used to provide additional insight into heterogeneous neuromuscular disease phenotypes and responses to treatment (Karlsson et al., 2019; Smith et al., 2017). Our work suggests that a refined TTNCS system with a simplified user interface could be valuable to clinicians. Outcomes further indicate that education and training in TTNCSs would be important accompaniments en route to broader use of the technique.

Funding

Blavatnik Sensory Disorders Research Award – Blavantnik Family Fund. The sponsor played no role in the study design, collection and interpretation of data, writing the report, or the decision to submit the article for publication.

Declaration of interests

None.

Acknowledgements

We wish to acknowledge the Blavatnik Family Fund. This work was done with the support of the Blavatnik Sensory Disorders Research Award (grant number 362283).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cnp.2022.08.003.

References

Bostock, H., Cikurel, K., Burke, D., 1998. Threshold tracking techniques in the study of human peripheral nerve. Muscle Nerve 21 (2), 137–158. https://doi.org/10.1002/(sici)1097-4598(199802)21:2<137::aid-mus1>3.0.co;2-c.

Burakgazi, A.Z., Messersmith, W., Vaidya, D., Hauer, P., Hoke, A., Polydefkis, M., 2011. Longitudinal assessment of oxaliplatin-induced neuropathy. Neurology 77 (10), 980–986. https://doi.org/10.1212/WNL.0b013e31822cfc59.

Chaudhry, V., Cornblath, D.R., Mellits, E.D., Avila, O., Freimer, M.L., Glass, J.D., Reim, J., Ronnett, G.V., Quaskey, S.A., Kuncl, R.W., 1991. Inter- and intra-examiner reliability of nerve conduction measurements in normal subjects. Ann. Neurol. 30 (6), 841–843.

Chaudhry, V., Corse, A.M., Freimer, M.L., Glass, J.D., Mellits, E.D., Kuncl, R.W., Quaskey, S.A., Cornblath, D.R., 1994. Inter- and intraexaminer reliability of nerve conduction measurements in patients with diabetic neuropathy. Neurology 44 (8).

Colloca, L., 2019. The Placebo effect in pain therapies. Annu. Rev. Pharmacol. Toxicol. 59, 191–211. https://doi.org/10.1146/annurev-pharmtox-010818-021542.

Diester, I., Hefti, F., Mansuy, I., Pascual-Leone, A., Robbins, T.W., Rubin, L.L., et al., 2016. Translational Neuroscience. MIT Press, Cambridge.

Dyck, P.J., Kratz, K.M., Lehman, K.A., Karnes, J.L., Melton, L.J., O'Brien, P.C., Litchy, W. J., Windebank, A.J., Smith, B.E., Low, P.A., Service, F.J., Rizza, R.A., Zimmerman, B. R., 1991. The Rochester Diabetic Neuropathy Study: design, criteria for types of neuropathy, selection bias, and reproducibility of neuropathic tests. Neurology 41 (6).

Emery, E.C., Young, G.T., Berrocoso, E.M., Chen, L., McNaughton, P.A., 2011. HCN2 ion channels play a central role in inflammatory and neuropathic pain. Science 333 (6048), 1462–1466. https://doi.org/10.1126/science.1206243.

Karlsson, P., Hincker, A.M., Jensen, T.S., Freeman, R., Haroutounian, S., 2019. Structural, functional, and symptom relations in painful distal symmetric polyneuropathies: a systematic review. Pain 160 (2), 286–297. https://doi.org/10.1097/j.pain.0000000000001381.

Kiernan, M.C., Burke, D., Andersen, K.V., Bostock, H., 2000. Multiple measures of axonal excitability: a new approach in clinical testing. Muscle Nerve 23 (3), 399–409. https://doi.org/10.1002/(sici)1097-4598(200003)23:3<399::aid-mus12>3.0.co;2-g.

Kiernan, M.C., Lin, C.S., Andersen, K.V., Murray, N.M., Bostock, H., 2001. Clinical evaluation of excitability measures in sensory nerve. Muscle Nerve 24 (7), 883–892. https://doi.org/10.1002/mus.1085.

Kiernan, M.C., Bostock, H., Park, S.B., Kaji, R., Krarup, C., Krishnan, A.V., Kuwabara, S., Lin, C.-Y., Misawa, S., Moldovan, M., Sung, J., Vucic, S., Wainger, B.J., Waxman, S., Burke, . Measurement of axonal excitability: Consensus guidelines. Clin. Neurophysiol. 131 (1), 308–323.

Koulouris, A.E., Edwards, R.R., Dorado, K., Schreiber, K.L., Lazaridou, A., Rajan, S., White, J., Garcia, J., Gibbons, C., Freeman, R., 2020. Reliability and validity of the Boston bedside quantitative sensory testing battery for neuropathic pain. Pain Med. 21 (10), 2336–2347.

Krishnan, A.V., Lin, C.S., Park, S.B., Kiernan, M.C., 2008. Assessment of nerve excitability in toxic and metabolic neuropathies. J. Peripher. Nerv. Syst. 13 (1), 7–26. https://doi.org/10.1111/j.1529-8027.2008.00155.x.

Lauria, G., Hsieh, S.T., Johansson, O., Kennedy, W.R., Leger, J.M., Mellgren, S.I., Nolano, M., Merkies, I.S.J., Polydefkis, M., Smith, A.G., Sommer, C., Valls-Solé, J., 2010. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. Eur. J. Neurol. 17 (7), 903–e49.

Salerno, D.F., Werner, R.A., Albers, J.W., Becker, M.P., Armstrong, T.J., Franzblau, A., 1999. Reliability of nerve conduction studies among active workers. Muscle Nerve 22 (10), 1372–1379. https://doi.org/10.1002/(sici)1097-4598(199910)22:10<1372::aid-mus6>3.0.co;2-s.

Smith, S.M., Dworkin, R.H., Turk, D.C., Baron, R., Polydefkis, M., Tracey, I., Borsook, D., Edwards, R.R., Harris, R.E., Wager, T.D., Arendt-Nielsen, L., Burke, L.B., Carr, D.B., Chappell, A., Farrar, J.T., Freeman, R., Gilron, I., Goli, V., Haeussler, J., Jensen, T., Katz, N.P., Kent, J., Kopecky, E.A., Lee, D.A., Maixner, W., Markman, J.D., McArthur, J.C., McDermott, M.P., Parvathenani, L., Raja, S.N., Rappaport, B.A., Rice, A.S.C., Rowbotham, M.C., Tobias, J.K., Wasan, A.D., Witter, J., 2017. The potential role of sensory testing, skin biopsy, and functional brain imaging as biomarkers in chronic pain clinical trials: IMMPACT considerations. J. Pain 18 (7), 757–777.

Tomlinson, S., Burke, D., Hanna, M., Koltzenburg, M., Bostock, H., 2010. In vivo assessment of HCN channel current (I(h)) in human motor axons. Muscle Nerve 41 (2), 247–256. https://doi.org/10.1002/mus.21482.

Tracey, I., Woolf, C.J., Andrews, N.A., 2019. Composite pain biomarker signatures for objective assessment and effective treatment. Neuron 101 (5), 783–800. https://doi.org/10.1016/j.neuron.2019.02.019.

Wainger, B.J., Macklin, E.A., Vucic, S., McIlduff, C.E., Paganoni, S., Maragakis, N.J., Bedlack, R., Goyal, N.A., Rutkove, S.B., Lange, D.J., Rivner, M.H., Goutman, S.A., Ladha, S.S., Mauricio, E.A., Baloh, R.H., Simmons, Z., Pothier, L., Kassis, S.B., La, T., Hall, M., Evora, A., Klements, D., Hurtado, A., Pereira, J.D., Koh, J., Celnik, P.A., Chaudhry, V., Gable, K., Juel, V.C., Phielipp, N., Marei, A., Rosenquist, P., Meehan, S., Oskarsson, B., Lewis, R.A., Kaur, D., Kiskinis, E., Woolf, C.J., Eggan, K., Weiss, M. D., Berry, J.D., David, W.S., Davila-Perez, P., Camprodon, J.A., Pascual-Leone, A., Kiernan, M.C., Shefner, J.M., Atassi, N., Cudkowicz, M.E., 2021. Effect of ezogabine on cortical and spinal motor neuron excitability in amyotrophic lateral sclerosis: A randomized clinical trial. JAMA Neurol. 78 (2), 186.

Weiss, M.D., Macklin, E.A., McIlduff, C.E., Vucic, S., Wainger, B.J., Kiernan, M.C., et al., 2021. Effects of mexiletine on hyperexcitability in sporadic amyotrophic lateral sclerosis: Preliminary findings from a small phase II randomized controlled trial. Muscle Nerve 63 (3), 371–383. https://doi.org/10.1002/mus.27146.