Hindawi Publishing Corporation
Physiology Journal
Volume 2013, Article ID 254789, 11 pages
http://dx.doi.org/10.1155/2013/254789

*Research Article*

# Noninvasive Evaluation of Nerve Conduction in Small Diameter Fibers in the Rat

**Elena G. Zotova[1] and Joseph C. Arezzo[1,2]**

[1] *The Saul R. Korey Department of Neurology, Albert Einstein College of Medicine, K322, 1300 Morris Park Avenue, Bronx, NY 10461, USA*
[2] *Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA*

Correspondence should be addressed to Elena G. Zotova; ezotova@optonline.net

Received 29 October 2012; Revised 27 December 2012; Accepted 28 December 2012

Academic Editor: Gary Lopaschuk



A novel noninvasive technique was applied to measure velocity within slow conducting axons in the distal extreme of the sciatic nerve (i.e., digital nerve) in a rat model. The technique is based on the extraction of rectified multiple unit activity (MUA) from *in vivo* whole nerve compound responses. This method reliably identifies compound action potentials in thinly myelinated fibers conducting at a range of 9–18 m/s (A$\delta$ axons), as well as in a subgroup of unmyelinated C fibers conducting at approximately 1-2 m/s. The sensitivity of the method to C-fiber conduction was confirmed by the progressive decrement of the responses in the 1-2 m/s range over a 20-day period following the topical application of capsaicin (ANOVA $P < 0.03$). Increasing the frequency of applied repetitive stimulation over a range of 0.75 Hz to 6.0 Hz produced slowing of conduction and a significant decrease in the magnitude of the compound C-fiber response (ANOVA $P < 0.01$). This technique offers a unique opportunity for the noninvasive, repeatable, and quantitative assessment of velocity in the subsets of A$\delta$ and C fibers in parallel with the evaluation of fast nerve conduction.

## 1. Introduction

The evaluation of peripheral nerve conduction velocity (NCV) (CAP: capsaicin, HFRS: high frequency repetitive stimulation, MUA: multiple unit activity, NCV: nerve conduction velocity, NFD: nerve fiber density, and SFN: small fiber neuropathy) is a well-established and highly utilized procedure for the assessment of the pattern, symmetry and extent of neuropathy in both pre-clinical and clinical studies [1–4]. When properly performed, NCV provides a sensitive, objective, and specific index of the onset and progression of multiple forms of nerve damage. C fibers make up the majority of fibers in some nerve segments; however, standard NCV measures are only sensitive to activity in fast conducting, heavily myelinated axons. The *in vivo* assessment of conduction in small diameter axons is a formidable task due to the requirement of high-threshold stimulation, asynchronous, slow conduction and phase cancellation with distance [5]. The inability to assess conduction, in thinly myelinated A$\delta$ axons or in unmyelinated C fibers is a severe limitation of NCV procedures. There is a growing recognition that many forms of idiopathic small fiber neuropathies (SFNs) may have been poorly diagnosed and their prevalence underestimated due, in part, to a lack of sensitive, noninvasive measures of this condition [5–10]. SFN can result in pain, paresthesias, abnormal thermal sensitivity, and various autonomic dysfunctions. Emerging data suggests that SFNs have unique etiologies and distinct genetic contributions and that they potentially differ in their response to therapeutic approaches [11–24].

The clinical diagnosis of SFN is generally based on symptoms or the presence of specific sensory deficits revealed by questionnaires and/or physical examination (e.g., diminished perception of "sharp"). These measures are subjective and often highly variable. The addition of epidermal fiber density has added sensitivity to the assessment of SFNs; however, the interpretation of these findings is often limited by wide ranges of "normal values" and differences in fiber counts as a function of location [25–37].

The situation is even more difficult in preclinical models of SFN, which generally are forced to rely on behavioral

assessment as a measure of small fiber dysfunction [38–45]. The behaviors in question (e.g., thermal withdrawal reaction) are often complex; they involve both CNS and PNS functions, and they can be influenced by learning and motivation. Although neuropathologic evaluation can be applied to animal models, the accurate evaluation of structural deficits in C fibers requires invasive, time-consuming, teased fiber techniques [11, 14, 46, 47]. Epidermal fiber density measures have also been applied to preclinical studies, but the sensitivity of the procedures has often been limited by the same high variability issues seen in the clinic [48–52]. Single unit recording provides the most complete and specific evaluation of the function of slow conducting fibers. These studies are critical for defining cellular and molecular mechanisms of SFN, but they require complicated, invasive procedures often restricted to the proximal segments of the nerves and have limited applications in assessment of the disease progression and/or in screening of potential therapeutics [53–63]. Microneurographic studies have reported that painful SFNs are often associated with an increase in spontaneous activity and changes in excitability of C fibers [64–67]. The introduction of microneurography to animal modeling confirmed similar patterns of C-fiber activity in rats and humans in both normal and pathological conditions; however, the accurate tracing of C-fiber activity in animal models required exposing the sciatic nerve, precluding longitudinal assessments [68, 69].

The present study was designed to define a reliable experimental technique for the assessment of slow conducting axons. The approach differs from the well-established use of single axon recording in several important ways: (1) it assesses whole nerve compound responses, allowing quantitative evaluation of the effects of experimental treatment or disease on both fast and slow conducting axons in parallel; (2) it is noninvasive, permitting repeat measurement of the slow nerve conduction as deficits progress and possibly recover; (3) it is a relatively simple procedure that could be implemented, in most cases, with existing electrophysiologic equipment. Application of this technique could augment the specificity of microneurography with longitudinal and quantitative assessment of activity in the whole intact nerve.

## 2. Materials and Methods

*2.1. Animals.* Electrophysiological studies were performed on 15 Sprague-Dawley female rats (Charles River Lab., Wilmington, MA) that were 5-6 months old at the start of the study. All animals were given free access to standard laboratory chow and water throughout the study. Five rats were treated with the topical application of capsaicin (CAP). The magnitude of C-fiber conduction in these rats were compared to that determined in 5 age-matched control rats at baseline (prior to the treatment), 10, 20, and 30–45 days after the administration of CAP. The remaining rats ($N = 5$) were used to help optimize the recording procedures and analysis. The use of all animals and procedures was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Institute Care and Use Committee at Albert Einstein College of Medicine.

*2.2. Electrophysiological Procedures.* All experiments were done under general anesthesia (isoflurane 1.5–2.5%/$O_2$), with the rats positioned on a heating pad and monitored for respiration and rectal temperature. Evoked activity was recorded in the digital nerve using subcutaneous platinum needle electrodes (Grass Astro-Med, Inc., Warwick, RI) for both recording and stimulation. The digital nerve was stimulated at the dorsal-superficial surface of the third cuneiform/proximal end of metatarsal (Figure 1, S1). A distal set of recording electrodes was positioned on the lateral side of the third toe (Figure 1, R1) 20 mm distal from the stimulation point. Another set of recording electrodes was positioned at the sciatic notch (75–85 mm proximally to the stimulation point) to monitor nerve conduction over the proximal segment of the nerve (Figure 1, R2). After evaluation of the proximal nerve conduction, percutaneous injection of 0.1–0.3 mL of 2% lidocaine (SPARHAWK, Laboratories, Inc., Lenexa, KS) was applied between the recording sites to block nerve conduction [70] and prevent the masking of slow conducting responses by central reflexes (e.g., dorsal root reflexes). The effective blocking of conduction in fast fibers was verified by the absence of neural response at the proximal site and monitored throughout the experiment. Responses were evoked by constant current electrical stimulation (Grass stimulator-11, isolation unit SIU-7, Warwick, RI), which was systematically increased in intensity (0.1–15 mA and duration 0.01–3.0 ms) until the magnitude of the C-fiber signal (i.e., velocities <2.0 m/s) was maximal.

The effects of high frequency repetitive stimulation (HFRS) on C-fiber activity were explored using stimulation rates of 0.75, 1.5, 3.0, and 6.0 Hz. The use of this procedure has been previously described for the evaluation of fast conducting fibers [71]. HFRS was conducted in step-like order from the lowest (0.75 Hz) to the highest (6.0 Hz) frequency. Prior to each escalation in frequency, a 0.75 Hz signal was evaluated to test the return of the slow fiber neural activity to the baseline level. Neuroelectric signals were impedance-matched and differentially amplified with a gain of 20,000 and a frequency band of 20 Hz–3 kHz using a BIOPAC MP100 and AcqKnowledge software version 3.7.2 (BIOPAC Systems Inc., Goleta, CA). Data were scanned for artifacts, digitized at the rate of 20 kHz, and averaged across 50–100 stimuli for slow nerve conduction and 5–20 stimuli for the fast nerve conduction. Single sweeps of slow nerve conduction were stored in a digital format (MDI DT-1600) for further off-line analysis.

*2.3. Multiple Unit Activity (MUA).* Measures of MUA were extracted from single sweep recording by first high-pass filtering of the response above 450 Hz and then full-wave rectifying of the signal prior to averaging [72–74]. Rectification minimizes phase cancellation of the asynchronous neural signals of slow conduction during averaging. MUA data represent composite voltages of the averaged whole nerve responses digitized at a constant sampling rate (i.e.,

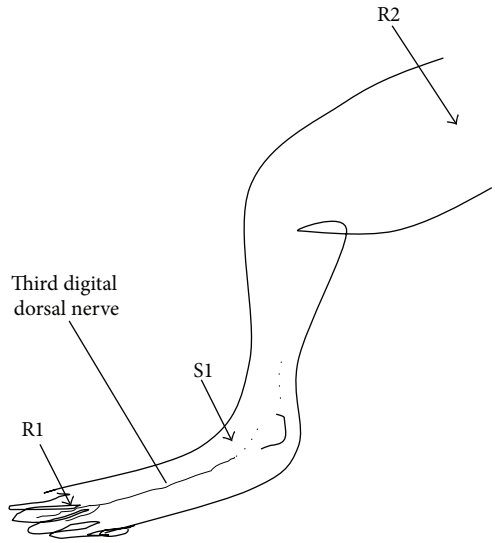


Figure 1: The schematic of electrode positions for electrophysiological recording over distal (R1) and proximal (R2) segments of the digital nerve with respect to stimulation (S1).

voltage per time bin). At the specific latencies over a fixed distance from the site of stimulation, these responses were integrated across a specific time period to reflect the signal strength in a specific range of velocities (i.e., 1 to 2 m/s). To balance the difference between the time periods of faster and slower velocities (e.g., 3 ms for 1.5–2 m/s and 20 ms for 0.5–1 m/s), the response integrated over each range of NCV was divided by the number of time bins (voltage samplings) within this range. The integrated MUA signal was expressed as a magnitude of response over the range of 0.5–2 m/s with respect to the level of spontaneous activity in the prestimulation epoch [75, 76].

*2.4. Application of Capsaicin.* Local skin denervation was induced by topical application of 0.1% CAP crème (Capzasin-HP, Chattem, Inc., Chattanooga, TN) to the third toe of rat hind paw. CAP was applied under continuous light isoflurane anesthesia (0.5%–1.0% in 0.3 L/min flow of $O_2$) for 10 hours/day over a four-day period. This procedure minimized discomfort of the animal during CAP application and assured consistency of treatment across animals. After each daily application, the residual crème was cleaned from the skin. The first post-CAP electrophysiologic measures occurred 10 days after discontinuation of CAP treatment; at that time, there was no evidence of inflammation or skin redness.

*2.5. Statistics.* Comparisons across conditions utilized standard inferential procedures (i.e., ANOVA); alpha levels were set at 0.05. If warranted, a Tukey's Multiple Comparison Test was used for cross-sectional evaluations. Experimental data are presented as mean ± SD, unless otherwise indicated.

## 3. Results

*3.1. Distal Digital Nerve Electrophysiology.* The patterns of digital nerve activity varied as a function of stimulus intensity and distal-to-proximal recording site. The lowest intensity evaluated (5 mA, 0.01 ms) uniquely activated low threshold axons and resulted in a composite response dominated by velocities over 55 m/s in the proximal nerve segments and between 26 and 42 m/s at the distal segments (Figure 2(a)). Increasing the stimulation intensity (10–15 mA-0.01–0.05 ms) added the activation of slower conduction fibers with velocities less than 20 m/s (semilate responses, Figure 2(a)). In the distal segment of digital nerve, maximal NCV was 35.1 ± 6.1 m/s with an amplitude of 11.6 ± 4.4 $\mu$V; the NCV of semilate responses was evaluated by peak latency and varied between 9.0 and 18.2 m/s (first peak mean NCV—16.3 ± 1.6 m/s). Simultaneously recorded neural response at the sciatic notch (proximal segment) demonstrated maximal NCV of 59.1 ± 4.2 m/s reflecting conduction in A$\alpha\beta$ fibers. These measurements are in agreement with previously published evaluations of maximal NCV and single unit recordings [62, 77–79] as well as histological evaluations of the morphometric fiber composition of the digital nerve as a distal branch of superficial peroneal nerve [80].

The activation of C fibers with associated conduction velocities less than 2.0 m/s required the use of high intensity stimulation (15 mA, 0.5–2 ms, Figure 2(b)). These stimulation currents and durations produced artifactual contamination of the earliest responses, and therefore the analysis of C-fiber activity was limited to the later MUA signals. The maximum magnitude of the slow NCV was observed in the range of 1-2 m/s, with the peak of activity in a range of 1.5–1.7 m/s (Figure 3). As expected, C-fiber responses extracted from whole nerve recordings were small and variable across animals (Figure 3(a)). Data recorded at a fixed distance from the stimulating cathode were averaged across subjects to improve the signal to noise ratio for the low amplitude, slow conducting responses. MUA was integrated across fixed time windows corresponding to velocities in the ranges of 2.0–1.6 m/s, 1.5–1.1 m/s, and 1.0–0.5 m/s and compared to the strength of the signal prior to stimulation. As a result, each compound C-fiber response was evaluated for a total magnitude of activity and its distribution in the range of velocities from 0.5 to 2 m/s (Figures 3(b) and 3(c)).

*3.2. Effects of Topical CAP.* The topical application of CAP resulted in a progressive decrease in the magnitude of the C-fiber compound response in the digital nerve over first 20 days after the end of treatment. Figure 4 illustrates the effects of CAP-induced denervation for responses with velocities in the C-fiber range. The effects of CAP are clearly seen in MUA averaged across 5 rats at four time points (Figure 4(a)). Evaluation of total activity of C fibers in the range of slow NCV (0.5–2 m/s) across time points confirmed a statistically significant decrease in activity (Figure 4(b), ANOVA $P < 0.03$). In addition to a decrease in total magnitude of the response, C fibers also demonstrated redistribution of MUA with a shift towards a slower range of velocities at the early stage of denervation. Untreated age-matched controls demonstrated no change in the same measures of C-fiber activity. The recovery of slow NCV was observed in a period of 30–45 days after drug application.

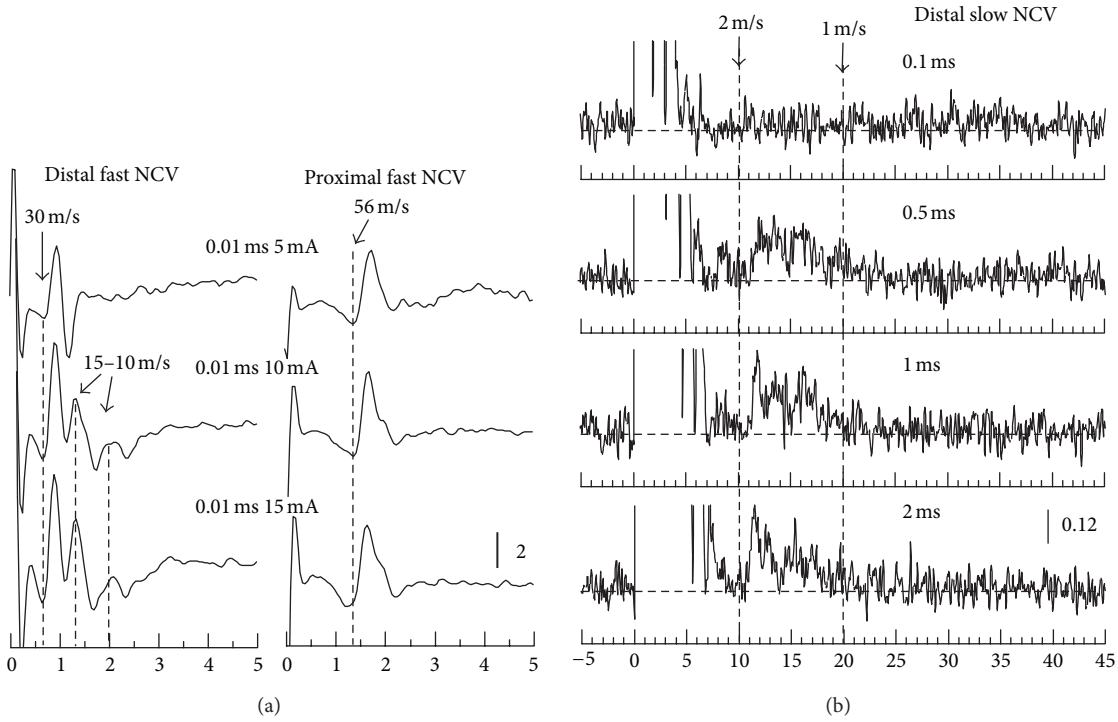


Figure 2: Representative traces of evoked potentials (a) and MUA (b) of a digital nerve are displayed as a function of intensity of stimulation. Numbers in the middle represent duration of the stimulus (ms) and intensity of constant current (mA). In (b), all recordings were done at constant current of 15 mA, and response of fast conduction is left out of scale (initial 5 ms after start of stimulation). Here and in the following figures, timescale is given in milliseconds and calibration is given in microvolts. Vertical dash lines indicate latencies for the marked NCV (m/s). Horizontal dash lines denote the level of activity in prestimuli interval.

*3.3. Effects of HFRS.* Conduction in C-fiber activity was clearly altered by increasing frequency rates. The initial decline in the magnitude of the C-fiber activity was evident with an HFRS of 1.5 Hz; at a rate of 6.0 Hz, the C-fiber response had been reduced by approximately 50% (ANOVA $P < 0.01$) compared to responses to low rates of stimulation (Figure 5(a)). In parallel with changes in magnitude, the distribution of velocities in the C-fiber range was also shifted to slower response as a function of increase in stimulation frequency (Figure 5(c)). Surprisingly, C-fiber response recorded at the frequency of 0.75 Hz right after HFRS demonstrated minimal or no decline in total MUA (Figure 5(b), 0 min), while a recording after a 5 min rest period showed a significant reduction in magnitude (Figure 5(b), 5 min, ANOVA $P < 0.01$). This paradox appears more prominent at the highest rates of stimulation (e.g., 3 and 6 Hz) and is most likely related to the slowing of A$\delta$ fibers into the range of C-fiber activity under the pressure of the elevated rates of stimulation. Immediately after termination of HFRS, the responses of some A$\delta$ fibers may still be delayed and cause an increase in compound response within the range of C-fiber conduction, while after 5 min rest, they recover and shift back to the shorter latencies, leaving the compound C-fiber response at the level of the magnitude comparable to the one during HFRS. Figure 5(d) provides an example of changes in representative traces of the distal A$\beta$ and A$\delta$ responses under conditions of HFRS. The slowest subset of A$\delta$ fibers (NCV range of 3-4 m/s) diminished the amplitude of responses during HFRS of 3–6 Hz and, therefore, may slow down into the range of conduction less than 2 m/s. As expected, the population of faster A$\delta$ fibers (e.g., NCV range of 7–12 m/s) demonstrated better resistance to the elevation of frequency and only slightly slowed conduction at the frequency of 6 Hz, while response in A$\beta$ fibers (peak NCV—17 m/s) did not change at any frequencies applied.

## 4. Discussion

The application of MUA techniques to *in vivo* electrophysiological measures provides an opportunity to measure the magnitude and range of NCV in C fibers in parallel with the assessment of conduction in the faster A$\beta$ and A$\delta$ fibers. This approach can identify velocities less than 2 m/s and can be applied repeatedly to the vulnerable distal segments of specific nerves. In the present study, up to 85% of C-fiber activity was recorded in the range of NCV between 1 and 2 m/s. The results are comparable to those observed in microneurographic recordings in humans [66, 81–83]. However, single unit studies, especially with the use of teased fiber preparation, can identify C-fiber velocities less than 1 m/s [54, 56, 60, 62, 63, 77, 84]. In the noninvasive, whole nerve approach used in the present study, the temporal dispersion and phase cancellation of activity in the slowest

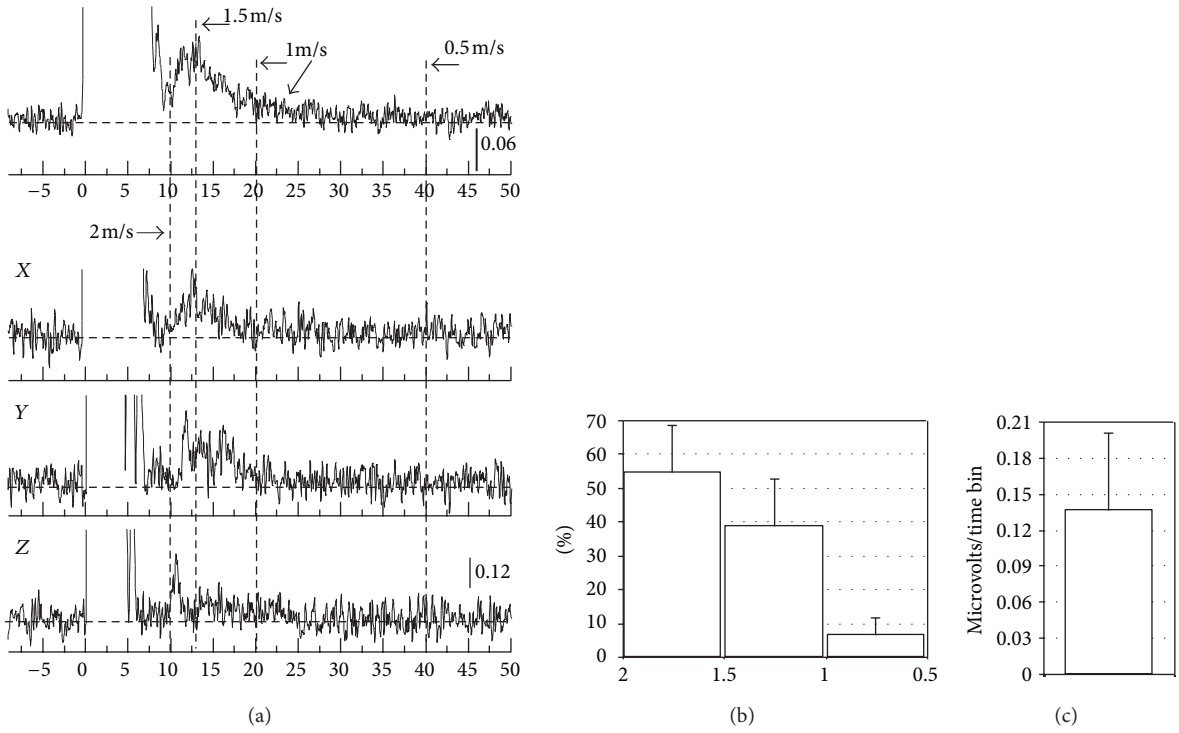


Figure 3: Compound C-fiber response is presented as MUA in digital nerve averaged across 15 animals ((a), top trace) and compared to the samples from three rats ($X$, $Y$, and $Z$). Response in the range of fast conduction is left out of scale (initial 5–7 ms after start of stimulation). Bar graphs illustrate distribution of activity within three ranges of slow NCV (horizontal axis, (b)) as a percent of the total response and magnitude of the C-fiber compound response (c) within the range of NCV 0.5–2 m/s (mean, SD).

subset of the C fibers may simply be too great for the summed response to significantly exceed prestimulus baseline levels. In addition, the use of stimulation of 0.75 Hz and above in the present study may be too rapid for activation of the slowest fibers, which in the single fiber studies are driven at the rate of 0.25–0.5 Hz [55, 63, 68, 82]. The increased sensitivity to activity in the subset of neurons conducting slower than 1 m/s in the single fiber techniques is gained at the expense of the sampling of a limited number of axons and/or the need for mechanical manipulation of the tissue (e.g., teased fiber preparation) which is generally performed at the more proximal nerve segments.

CAP selectively affects distal endings of A$\delta$ and C fibers [85]. Prolonged topical application of CAP leads to local skin desensitization and depletion of peripheral endings of small diameter fibers evident by immunohistochemical markers [86–91]. We employed CAP to confirm that MUA in the range of slow NCV originates from the activity in C fibers and to explore the ability of new electrophysiological evaluation to register small fiber degeneration and regeneration. The compound C-fiber response was significantly reduced during the first 20 days after CAP application and recovered in 30–45 days. These functional measures were consistent with the results observed in clinic for the same population of axons evaluated by epidermal and subepidermal nerve fiber density (NFD) and quantitative sensory testing [90, 91]. In humans, a reduction of NFD in the skin was observed in 2–7 days after topical CAP application, while initial reinnervation of CAP-treated skin was marked between 20 and 40 days after application [91]. The longer time period needed for electrophysiological registration of denervations is most likely related to the greater extent of axonal degeneration which is required for the elimination of nerve compound response compared to the reduction of density of nerve endings in the skin and subcutaneous tissue. At the early stage of degeneration (10 days), C fibers demonstrated a redistribution of MUA toward a slower range of NCV, in addition to a decrease in total magnitude of the response. Similar signs of dysfunction were observed in distal diabetic autonomic neuropathy [76].

Assessment of activity-dependent changes is an important experimental tool for the characterization of C-fiber function. While fast conducting fibers can easily follow frequencies up to 100 Hz, slow conducting fibers are known for significant limitations in the ability to respond to rapid stimulation [53, 54, 57, 61, 63, 64, 66, 81–84, 92–96]. Using repetitive stimulation, conduction of C fibers is altered at rates of 1 Hz and, in some circumstances, blocked at frequencies greater than 2 Hz [81, 92, 97]. In the present study, HFRS altered C-fiber compound response in two ways: higher stimulation rates reduced the overall response magnitude of the C-fiber response and shifted the distribution of NCV to

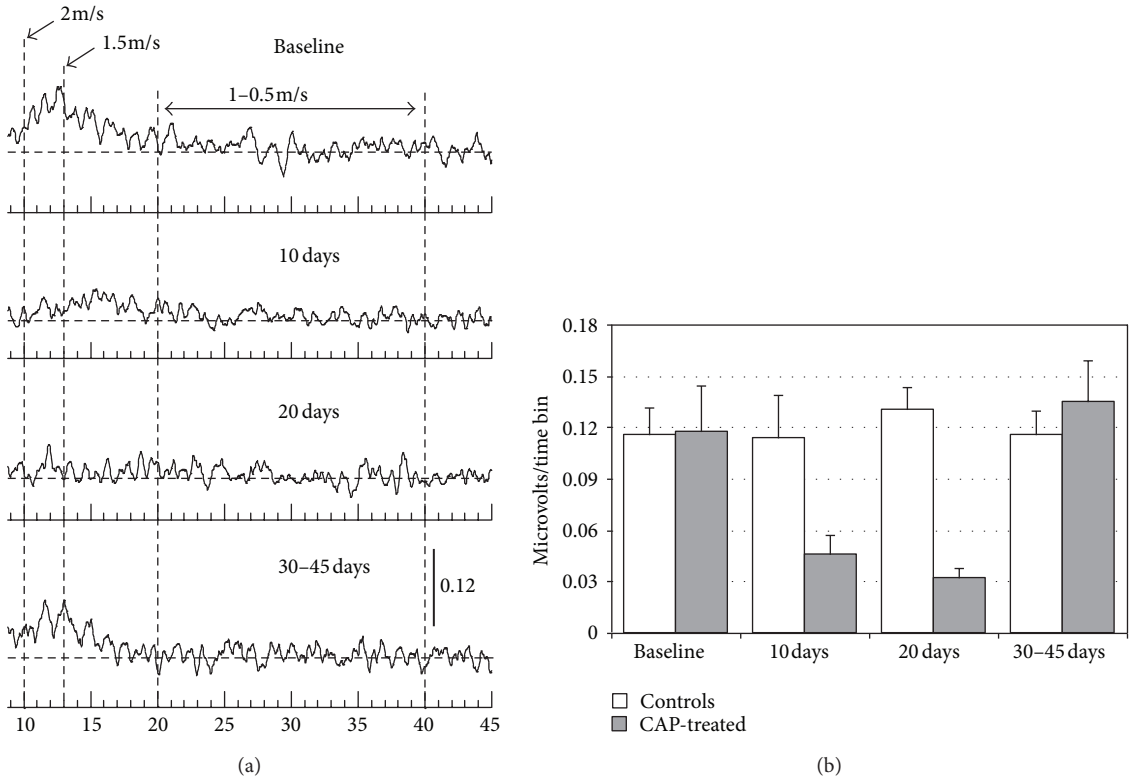


Figure 4: Effects of CAP application are reflected in the magnitude of compound C-fiber response in the digital nerve. (a) MUA was recorded prior to application of CAP (baseline) and 10, 20, and 30–45 days after application and averaged across 5 rats. (b) A decrease in total magnitude of the C-fiber compound response within the range of NCV 0.5–2 m/s after treatment with CAP (ANOVA $P < 0.03$, closed bars, mean, SEM, $N = 5$) is compared to no change of the same measure in age-matched controls (open bars, mean, SEM, $N = 5$). For illustration purposes the averaged MUA was smoothed.

slower values. The initial drop in magnitude of the compound response was observed at 1.5 Hz, while stimulation at 3 Hz increased the slowing of conduction. At a stimulation rate of 6 Hz, the C-fiber response was reduced by 60% compared to responses at the rate of 0.75 Hz, and the range of NCV has shifted from 1.5–2 m/s to less than 1.5 m/s.

The complex effects of stimulation rates in the present study support the contribution of a variety of unmyelinated fiber types to the compound C-fiber response. Single unit recordings confirm that high rates of repetitive stimulation are associated with progressive slowing, partial failure of conduction, and an increase in temporal dispersion in C fibers [68, 81, 92, 97–99]. The nature and severity of these effects also depend on the class/modality of activated C fibers [54, 84, 99]. For instance, mechano-insensitive C fibers are very sensitive to stimulation rates, while sympathetic efferent fibers demonstrated minimal changes in conduction under the same conditions. When measured as part of a composite whole nerve response, the effects on the various sub-types of C fibers can create complex patterns reflecting both slowing (e.g., redistribution of NCV) and blocking of slow conduction (e.g., reduction in magnitude of response), as it was observed in the compound responses of the current study.

Another difficulty in the assessment of slow conducting axons is the possible interaction of activity from overlapping populations of the slowest subset of A$\delta$ fibers and the fastest of the C fibers. With greater distances, these velocities would be clearly segregated, but when the examining patterns are isolated from the distal extremes of the whole nerve over a few centimeters, activity in these populations can be complex. A$\delta$ fibers demonstrate slowing and partial failure of conduction at much lower frequencies than A$\alpha$ and A$\beta$ fibers; activity in these fibers comes very close to the margins of C-fiber conduction in skin innervation [77, 92, 100, 101]. The subcomponents of the late responses can be explored using different time periods after HFRS. Relatively fast conducting subpopulations demonstrate minimal suppression following HFRS, while activity in slower C fibers can continue to be diminished for a period of up to 5 min after stimulation. Under the pressure of HFRS, some A$\delta$ fibers may slow down to the C-fiber range of velocities [100]; however, these larger diameter axons can be identified by their faster rate of recovery.

*4.1. Advantages and Limitations of the Novel Approach.* The advantages of the use of noninvasive measures of C-fiber activity are obvious. The outlined technique can reliably quantify activity in axons conducting at 1-2 m/s, the measures are relatively rapid, they can be repeated in the same nerve

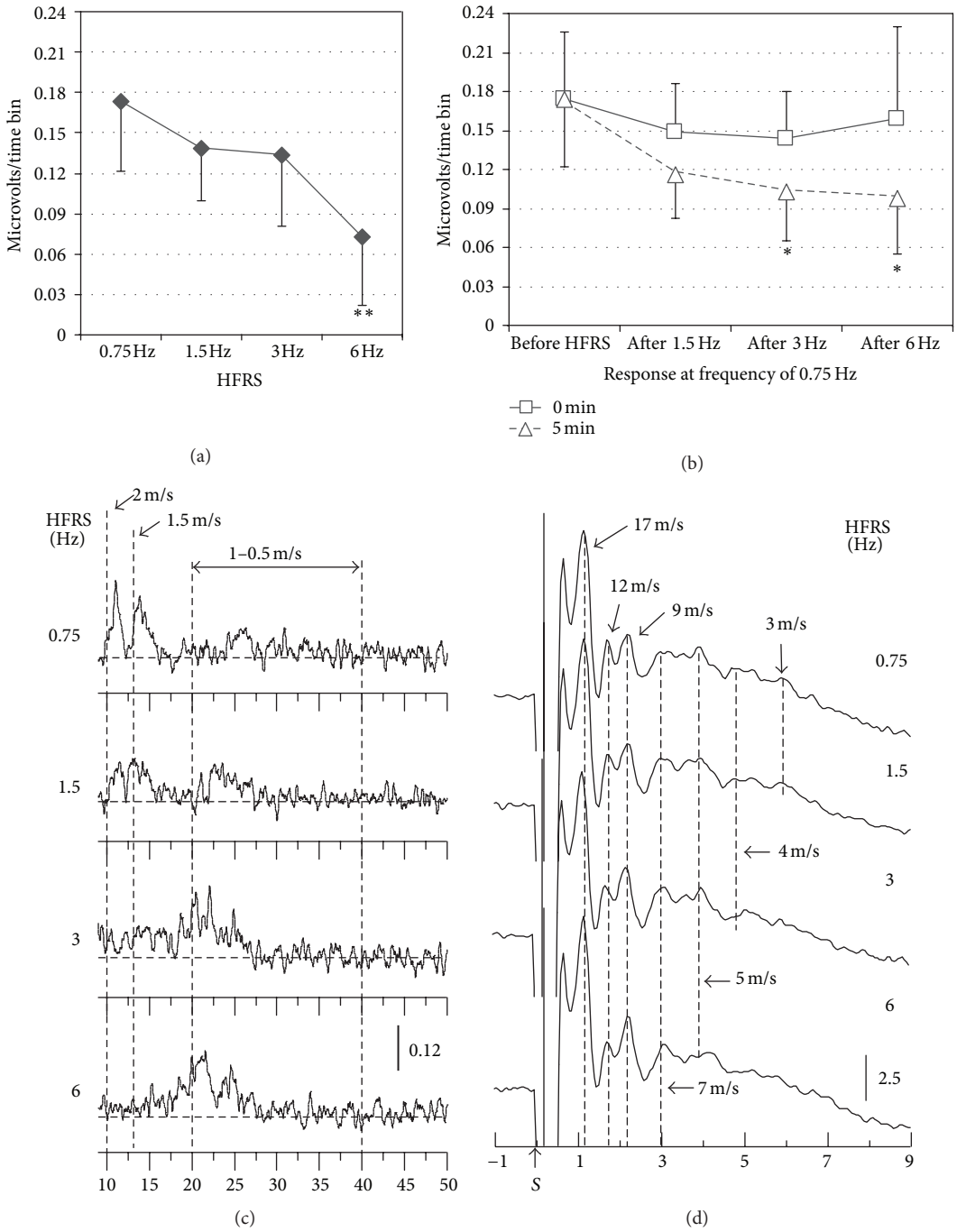


FIGURE 5: Changes in distal slow conduction under condition of HFRS: ((a) and (b)) total magnitude of C-fiber compound response (mean, SD, $N = 8$) during (a) and after HFRS ((b), 0 min—solid line, 5 min—dashed line); (c) and (d) demonstrate changes in representative traces of C-fiber compound response ((c), MUA) and in A$\beta$-A$\delta$ evoked potential (d) under conditions of HFRS. "S" next to the timescale marks start of stimulation. Asterisks denote statistically significant difference ($^{**}P < 0.01$, $^{*}P < 0.05$) between the magnitude of the initial response to frequency 0.75 Hz and responses during and after HFRS.

segment in longitudinal studies, and the procedure can measure conduction in fast and slow axons in the same experiment. As expected, the compound measure of slow conduction is sensitive to the effects of CAP, and subcomponents of slow conducting fibers can be differentiated by their response to stimulation rate and their fatigue. In contrast to the single unit assessments, the obtained results reflect the activity of the population of C fibers within intact nerves. The measures are parametric and subject to statistical comparison across time and experimental conditions. The limitations of this technique are also clear. The activation of high-threshold C fibers requires intense stimulation which

may limit this technique to anesthetized preparation. The composite nature of the C-fiber response confounds the identification of activity in specific subpopulations, although this can be facilitated by use of HFRS and recovery profiles. As measured in this study, the compound response is insensitive to conduction below 1 m/s, but this may be altered by the use of lower stimulation rates and a higher resolution averaging.

The relatively simple evaluation of C-fiber conduction outlined in the study offers a novel tool for examining unique electrophysiological aspects of SFN and may complement structural measures of skin biopsy with functional evaluation of deficits in transmembrane ion distribution, nodal integrity, and energy utilization (mitochondrial function) acquired from the whole nerve response of small diameter nerve fibers. The longitudinal measure of conduction in A$\delta$ and C fibers will bridge the gap between discoveries on the axonal level and their preclinical evaluation by gross behavior (e.g., withdrawal reaction).

## Conflict of Interests

The authors report no conflict of interests.

## Acknowledgments

The authors would like to thank Shirley Seto and Linda O'Donnell for their assistance in preparation of the paper. This work was supported by NIH Grant R21 RR025896.

## References

[1] J. C. Arezzo, "New developments in the diagnosis of diabetic neuropathy," *American Journal of Medicine*, vol. 107, no. 2, pp. 9S–16S, 1999.

[2] O. Boyraz and M. Saracoglu, "The effect of obesity on the assessment of diabetic peripheral neuropathy: a comparison of Michigan patient version test and Michigan physical assessment," *Diabetes Research and Clinical Practice*, vol. 90, no. 3, pp. 256–260, 2010.

[3] G. Cavaletti and P. Marmiroli, "Chemotherapy-induced peripheral neurotoxicity," *Nature Reviews Neurology*, vol. 6, no. 12, pp. 657–666, 2010.

[4] P. J. Dyck, J. W. Albers, H. Andersen et al., "Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity," *Diabetes/Metabolism Research and Reviews*, vol. 27, no. 7, pp. 620–628, 2011.

[5] J. C. Arezzo and E. Zotova, "Electrophysiologic measures of diabetic neuropathy: mechanism and meaning," *International Review of Neurobiology*, vol. 50, pp. 229–255, 2002.

[6] D. Lacomis, "Small-fiber neuropathy," *Muscle and Nerve*, vol. 26, no. 2, pp. 173–188, 2002.

[7] E. Fink and A. L. Oaklander, "Small-fiber neuropathy: answering the burning questions," *Science of Aging Knowledge Environment*, vol. 2006, no. 6, article pe7, 2006.

[8] E. L. Feldman, D. R. Cornblath, J. Porter, R. Dworkin, and S. Scherer, "National Institute of Neurological Disorders and Stroke (NINDS): advances in understanding and treating neuropathy, 24-25 October 2006; Bethesda, Maryland," *Journal of the Peripheral Nervous System*, vol. 13, no. 1, pp. 1–6, 2008.

[9] J. C. Arezzo, M. S. Litwak, and E. G. Zotova, "Correlation and dissociation of electrophysiology and histopathology in the assessment of toxic neuropathy," *Toxicologic Pathology*, vol. 39, no. 1, pp. 46–51, 2011.

[10] Y. So, "New insights into small fiber neuropathy," *Annals of Neurology*, vol. 71, no. 1, pp. 3–4, 2012.

[11] S. Yagihashi, M. Kamijo, Y. Ido, and D. J. Mirrlees, "Effects of long-term aldose reductase inhibition on development of experimental diabetic neuropathy. Ultrastructural and morphometric studies of sural nerve in streptozocin-induced diabetic rats," *Diabetes*, vol. 39, no. 6, pp. 690–696, 1990.

[12] A. J. M. Boulton, A. I. Vinik, J. C. Arezzo et al., "Diabetic neuropathies: a statement by the American Diabetes Association," *Diabetes Care*, vol. 28, no. 4, pp. 956–962, 2005.

[13] S. J. L. Flatters and G. J. Bennett, "Studies of peripheral sensory nerves in paclitaxel-induced painful peripheral neuropathy: evidence for mitochondrial dysfunction," *Pain*, vol. 122, no. 3, pp. 245–257, 2006.

[14] S. C. Keswani, C. Jack, C. Zhou, and A. Höke, "Establishment of a rodent model of HIV-associated sensory neuropathy," *Journal of Neuroscience*, vol. 26, no. 40, pp. 10299–10304, 2006.

[15] J. J. Lee, J. A. Low, E. Croarkin et al., "Changes in neurologic function tests may predict neurotoxicity caused by ixabepilone," *Journal of Clinical Oncology*, vol. 24, no. 13, pp. 2084–2091, 2006.

[16] R. M. Herman, J. B. Brower, D. G. Stoddard et al., "Prevalence of somatic small fiber neuropathy in obesity," *International Journal of Obesity*, vol. 31, no. 2, pp. 226–235, 2007.

[17] A. I. Vinik and D. Ziegler, "Diabetic cardiovascular autonomic neuropathy," *Circulation*, vol. 115, no. 3, pp. 387–397, 2007.

[18] A. Gonzalez-Duarte, J. Robinson-Papp, and D. M. Simpson, "Diagnosis and management of HIV-associated neuropathy," *Neurologic Clinics*, vol. 26, no. 3, pp. 821–832, 2008.

[19] H. W. Jin, S. J. L. Flatters, W. H. Xiao, H. L. Mulhern, and G. J. Bennett, "Prevention of paclitaxel-evoked painful peripheral neuropathy by acetyl-l-carnitine: effects on axonal mitochondria, sensory nerve fiber terminal arbors, and cutaneous Langerhans cells," *Experimental Neurology*, vol. 210, no. 1, pp. 229–237, 2008.

[20] S. Løseth, S. I. Mellgren, R. Jorde, S. Lindal, and E. Stalberg, "Polyneuropathy in type 1 and type 2 diabetes: comparison of nerve conduction studies, thermal perception thresholds and intraepidermal nerve fibre densities," *Diabetes/Metabolism Research and Reviews*, vol. 26, no. 2, pp. 100–106, 2010.

[21] P. Vivithanaporn, G. Heo, J. Gamble et al., "Neurologic disease burden in treated HIV/AIDS predicts survival: a population-based study," *Neurology*, vol. 75, no. 13, pp. 1150–1158, 2010.

[22] J. Tavee and D. Culver, "Sarcoidosis and small-fiber neuropathy," *Current Pain and Headache Reports*, vol. 15, no. 3, pp. 201–206, 2011.

[23] W. H. Xiao, H. Zheng, F. Y. Zheng, R. Nuydens, T. F. Meert, and G. J. Bennett, "Mitochondrial abnormality in sensory, but not motor, axons in paclitaxel-evoked painful peripheral neuropathy in the rat," *Neuroscience*, vol. 199, pp. 461–469, 2011.

[24] W. H. Xiao, H. Zheng, and G. J. Bennett, "Characterization of oxaliplatin-induced chronic painful peripheral neuropathy in the rat and comparison with the neuropathy induced by paclitaxel," *Neuroscience*, vol. 203, pp. 194–206, 2012.

[25] M. Polydefkis, P. Hauer, J. W. Griffin, and J. C. McArthur, "Skin biopsy as a tool to assess distal small fiber innervation in diabetic neuropathy," *Diabetes Technology and Therapeutics*, vol. 3, no. 1, pp. 23–28, 2001.

[26] E. Hoitsma, J. P. H. Reulen, M. de Baets, M. Drent, F. Spaans, and C. G. Faber, "Small fiber neuropathy: a common and important clinical disorder," *Journal of the Neurological Sciences*, vol. 227, no. 1, pp. 119–130, 2004.

[27] S. H. Horowitz, "The diagnostic workup of patients with neuropathic pain," *Anesthesiology Clinics*, vol. 25, no. 4, pp. 699–708, 2007.

[28] G. Devigili, V. Tugnoli, P. Penza et al., "The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology," *Brain*, vol. 131, no. 7, pp. 1912–1925, 2008.

[29] M. M. Backonja, D. Walk, R. R. Edwards et al., "Quantitative sensory testing in measurement of neuropathic pain phenomena and other sensory abnormalities," *The Clinical Journal of Pain*, vol. 25, no. 7, pp. 641–647, 2009.

[30] M. Nebuchennykh, S. Løseth, S. Lindal, and S. I. Mellgren, "The value of skin biopsy with recording of intraepidermal nerve fiber density and quantitative sensory testing in the assessment of small fiber involvement in patients with different causes of polyneuropathy," *Journal of Neurology*, vol. 256, no. 7, pp. 1067–1075, 2009.

[31] D. Walk, N. Sehgal, T. Moeller-Bertram et al., "Quantitative sensory testing and mapping: a review of nonautomated quantitative methods for examination of the patient with neuropathic pain," *The Clinical Journal of Pain*, vol. 25, no. 7, pp. 632–640, 2009.

[32] G. Cavaletti, B. Frigeni, F. Lanzani et al., "Chemotherapy-induced peripheral neurotoxicity assessment: a critical revision of the currently available tools," *European Journal of Cancer*, vol. 46, no. 3, pp. 479–494, 2010.

[33] G. Lauria, S. T. Hsieh, O. Johansson et al., "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," *Journal of the Peripheral Nervous System*, vol. 15, no. 2, pp. 79–92, 2010.

[34] A. Hlubocky, K. Wellik, M. A. Ross et al., "Skin biopsy for diagnosis of small fiber neuropathy: a critically appraised topic," *Neurologist*, vol. 16, no. 1, pp. 61–63, 2010.

[35] G. Lauria, M. Bakkers, C. Schmitz et al., "Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study," *Journal of the Peripheral Nervous System*, vol. 15, no. 3, pp. 202–207, 2010.

[36] S. I. Mellgren and S. Lindal, "Nerve biopsy—some comments on procedures and indications," *Acta Neurologica Scandinavica*, vol. 191, pp. 64–70, 2011.

[37] S. Mackey, I. Carroll, B. Emir, T. K. Murphy, E. Whalen, and L. Dumenci, "Sensory pain qualities in neuropathic pain," *Journal of Pain*, vol. 13, pp. 58–63, 2012.

[38] N. Authier, J. P. Gillet, J. Fialip, A. Eschalier, and F. Coudore, "Description of a short-term Taxol-induced nociceptive neuropathy in rats," *Brain Research*, vol. 887, no. 2, pp. 239–249, 2000.

[39] R. Baron, "Peripheral neuropathic pain: from mechanisms to symptoms," *Clinical Journal of Pain*, vol. 16, no. 2, pp. S12–S20, 2000.

[40] L. Gagliese and R. Melzack, "Age differences in nociception and pain behaviours in the rat," *Neuroscience and Biobehavioral Reviews*, vol. 24, no. 8, pp. 843–854, 2000.

[41] A. S. C. Rice, D. Cimino-Brown, J. C. Eisenach et al., "Animal models and the prediction of efficacy in clinical trials of analgesic drugs: a critical appraisal and call for uniform reporting standards," *Pain*, vol. 139, no. 2, pp. 243–247, 2008.

[42] K. A. Sullivan, S. I. Lentz, J. L. Roberts, and E. L. Feldman, "Criteria for creating and assessing mouse models of diabetic neuropathy," *Current Drug Targets*, vol. 9, no. 1, pp. 3–13, 2008.

[43] N. Authier, D. Balayssac, F. Marchand et al., "Animal models of chemotherapy-evoked painful peripheral neuropathies," *Neurotherapeutics*, vol. 6, no. 4, pp. 620–629, 2009.

[44] I. G. Obrosova, "Diabetic painful and insensate neuropathy: pathogenesis and potential treatments," *Neurotherapeutics*, vol. 6, no. 4, pp. 638–647, 2009.

[45] J. Sandkühler, "Models and mechanisms of hyperalgesia and allodynia," *Physiological Reviews*, vol. 89, pp. 707–758, 2009.

[46] A. I. Basbaum, M. Gautron, F. Jazat, M. Mayes, and G. Guilbaud, "The spectrum of fiber loss in a model of neuropathic pain in the rat: an electron microscopic study," *Pain*, vol. 47, no. 3, pp. 359–367, 1991.

[47] K. Sugimoto and S. Yagihashi, "Peripheral nerve pathology in rats with streptozotocin-induced insulinoma," *Acta Neuropathologica*, vol. 91, no. 6, pp. 616–623, 1996.

[48] G. Lauria, R. Lombardi, M. Borgna et al., "Intraepidermal nerve fiber density in rat foot pad: neuropathologic- neurophysiologic correlation," *Journal of the Peripheral Nervous System*, vol. 10, no. 2, pp. 202–208, 2005.

[49] C. Siau, W. Xiao, and G. J. Bennett, "Paclitaxel- and vincristine-evoked painful peripheral neuropathies: loss of epidermal innervation and activation of Langerhans cells," *Experimental Neurology*, vol. 201, no. 2, pp. 507–514, 2006.

[50] K. K. Beiswenger, N. A. Calcutt, and A. P. Mizisin, "Epidermal nerve fiber quantification in the assessment of diabetic neuropathy," *Acta Histochemica*, vol. 110, no. 5, pp. 351–362, 2008.

[51] H. T. Cheng, J. R. Dauch, J. M. Hayes, B. M. Yanik, and E. L. Feldman, "Nerve growth factor/p38 signaling increases intraepidermal nerve fiber densities in painful neuropathy of type 2 diabetes," *Neurobiology of Disease*, vol. 45, no. 1, pp. 280–287, 2012.

[52] A. Höke, "Animal models of peripheral neuropathies," *Neurotherapeutics*, vol. 9, no. 2, pp. 262–269, 2012.

[53] H. C. Shin, Y. L. Lee, H. Y. Kwon, H. J. Park, and S. A. Raymond, "Activity-dependent variations in conduction velocity of C fibers of rat sciatic nerve," *Neuroscience Research*, vol. 19, no. 4, pp. 427–431, 1994.

[54] M. D. Gee, B. Lynn, and B. Cotsell, "Activity-dependent slowing of conduction velocity provides a method for identifying different functional classes of C-fibre in the rat saphenous nerve," *Neuroscience*, vol. 73, no. 3, pp. 667–675, 1996.

[55] H. Bostock, M. Campero, J. Serra, and J. Ochoa, "Velocity recovery cycles of C fibres innervating human skin," *Journal of Physiology*, vol. 553, no. 2, pp. 649–663, 2003.

[56] X. Chen and J. D. Levine, "Altered temporal pattern of mechanically evoked C-fiber activity in a model of diabetic neuropathy in the rat," *Neuroscience*, vol. 121, no. 4, pp. 1007–1015, 2003.

[57] Y. B. Peng, M. Ringkamp, R. A. Meyer, and J. N. Campbell, "Fatigue and paradoxical enhancement of heat response in C-fiber nociceptors from cross-modal excitation," *Journal of Neuroscience*, vol. 23, no. 11, pp. 4766–4774, 2003.

[58] K. D. Tanner, D. B. Reichling, R. W. Gear, S. M. Paul, and J. D. Levine, "Altered temporal pattern of evoked afferent activity in a rat model of vincristine-induced painful peripheral neuropathy," *Neuroscience*, vol. 118, no. 3, pp. 809–817, 2003.

[59] L. Djouhri, S. Koutsikou, X. Fang, S. McMullan, and S. N. Lawson, "Spontaneous pain, both neuropathic and inflammatory, is related to frequency of spontaneous firing in intact C-fiber nociceptors," *Journal of Neuroscience*, vol. 26, no. 4, pp. 1281–1292, 2006.

[60] X. Chen and J. D. Levine, "Mechanically-evoked C-fiber activity in painful alcohol and AIDS therapy neuropathy in the rat," *Molecular Pain*, vol. 3, article 5, 2007.

[61] B. Y. Li, B. Feng, H. Y. Tsu, and J. H. Schild, "Unmyelinated visceral afferents exhibit frequency dependent action potential broadening while myelinated visceral afferents do not," *Neuroscience Letters*, vol. 421, no. 1, pp. 62–66, 2007.

[62] W. H. Xiao and G. J. Bennett, "Chemotherapy-evoked neuropathic pain: abnormal spontaneous discharge in A-fiber and C-fiber primary afferent neurons and its suppression by acetyl-l-carnitine," *Pain*, vol. 135, no. 3, pp. 262–270, 2008.

[63] T. Taguchi, H. Ota, T. Matsuda, S. Murase, and K. Mizumura, "Cutaneous C-fiber nociceptor responses and nociceptive behaviors in aged Sprague-Dawley rats," *Pain*, vol. 151, no. 3, pp. 771–782, 2010.

[64] M. Schmelz, "Translating nociceptive processing into human pain models," *Experimental Brain Research*, vol. 196, no. 1, pp. 173–178, 2009.

[65] J. Serra, R. Solà, C. Quiles et al., "C-nociceptors sensitized to cold in a patient with small-fiber neuropathy and cold allodynia," *Pain*, vol. 147, no. 1–3, pp. 46–53, 2009.

[66] K. Ørstavik and E. Jørum, "Microneurographic findings of relevance to pain in patients with erythromelalgia and patients with diabetic neuropathy," *Neuroscience Letters*, vol. 470, no. 3, pp. 180–184, 2010.

[67] R. Schmidt, I. P. Kleggetveit, B. Namer Helås T et al., "Double spikes to single electrical stimulation correlates to spontaneous activity of nociceptors in painful neuropathy patients," *Pain*, vol. 153, pp. 391–398, 2012.

[68] J. Serra, H. Bostock, and X. Navarro, "Microneurography in rats: a minimally invasive method to record single C-fiber action potentials from peripheral nerves in vivo," *Neuroscience Letters*, vol. 470, no. 3, pp. 168–174, 2010.

[69] J. Serra, H. Bostock, R. Solà et al., "Microneurographic identification of spontaneous activity in C-nociceptors in neuropathic pain states in humans and rats," *Pain*, vol. 153, no. 1, pp. 42–55, 2012.

[70] A. P. Gokin, B. Philip, and G. R. Strichartz, "Preferential block of small myelinated sensory and motor fibers by lidocaine: in vivo electrophysiology in the rat sciatic nerve," *Anesthesiology*, vol. 95, no. 6, pp. 1441–1454, 2001.

[71] E. G. Zotova, G. J. Christ, W. Zhao, M. Tar, S. D. Kuppam, and J. C. Arezzo, "Effects of fidarestat, an aldose reductase inhibitor, on nerve conduction velocity and bladder function in streptozotocin-treated female rats," *Journal of Diabetes and Its Complications*, vol. 21, no. 3, pp. 187–195, 2007.

[72] A. D. Legatt, J. Arezzo, and H. G. Vaughan Jr., "Averaged multiple unit activity as an estimate of phasic changes in local neuronal activity: effects of volume-conducted potentials," *Journal of Neuroscience Methods*, vol. 2, no. 2, pp. 203–217, 1980.

[73] J. C. Arezzo, H. G. Vaughan Jr., M. A. Kraut, M. Steinschneider, and A. D. Legatt, "Intracranial generators of event related potentials in the monkey," in *Frontiers of Clinical Neuroscience. Evoked Potentials*, R. Q. Cracco and I. Bodis Wollner, Eds., vol. 3, pp. 174–189, Alan R. Liss, New York, NY, USA, 1986.

[74] M. Steinschneider, Y. I. Fishman, and J. C. Arezzo, "Spectrotemporal analysis of evoked and induced electroencephalographic responses in primary auditory cortex (A1) of the awake monkey," *Cerebral Cortex*, vol. 18, no. 3, pp. 610–625, 2008.

[75] H. H. Schaumburg, E. Zotova, B. Cannella et al., "Structural and functional investigations of the murine cavernosal nerve: a model system for serial spatio-temporal study of autonomic neuropathy," *British Journal of Urology International*, vol. 99, no. 4, pp. 916–924, 2007.

[76] E. G. Zotova, H. H. Schaumburg, C. S. Raine et al., "Effects of hyperglycemia on rat cavernous nerve axons: a functional and ultrastructural study," *Experimental Neurology*, vol. 213, no. 2, pp. 439–447, 2008.

[77] G. R. Lewin and S. B. McMahon, "Physiological properties of primary sensory neurons appropriately and inappropriately innervating skin in the adult rat," *Journal of Neurophysiology*, vol. 66, no. 4, pp. 1205–1217, 1991.

[78] F. Li, O. I. Abatan, H. Kim et al., "Taurine reverses neurological and neurovascular deficits in Zucker diabetic fatty rats," *Neurobiology of Disease*, vol. 22, no. 3, pp. 669–676, 2006.

[79] H. H. Schaumburg, E. Zotova, C. S. Raine, M. Tar, and J. Arezzo, "The rat caudal nerves: a model for experimental neuropathies," *Journal of the Peripheral Nervous System*, vol. 15, no. 2, pp. 128–139, 2010.

[80] B. Povlsen, N. Stankovic, P. Danielsson, and C. Hildebrand, "Fiber composition of the lateral plantar and superficial peroneal nerves in the rat foot," *Anatomy and Embryology*, vol. 189, no. 5, pp. 393–399, 1994.

[81] J. Serra, M. Campero, J. Ochoa, and H. Bostock, "Activity-dependent slowing of conduction differentiates functional subtypes of C fibres innervating human skin," *Journal of Physiology*, vol. 515, no. 3, pp. 799–811, 1999.

[82] B. Namer, B. Barta, K. Ørstavik et al., "Microneurographic assessment of C-fibre function in aged healthy subjects," *Journal of Physiology*, vol. 587, no. 2, pp. 419–428, 2009.

[83] M. Schmelz and R. Schmidt, "Microneurographic single-unit recordings to assess receptive properties of afferent human C-fibers," *Neuroscience Letters*, vol. 470, no. 3, pp. 158–161, 2010.

[84] M. Ringkamp, L. M. Johanek, J. Borzan et al., "Conduction properties distinguish unmyelinated sympathetic efferent fibers and unmyelinated primary afferent fibers in the monkey," *PLoS ONE*, vol. 5, no. 2, article e9076, 2010.

[85] M. J. Caterina and D. Julius, "The vanilloid receptor: a molecular gateway to the pain pathway," *Annual Review of Neuroscience*, vol. 24, pp. 487–517, 2001.

[86] S. B. McMahon, G. Lewin, and S. R. Bloom, "The consequences of long-term topical capsaicin application in the rat," *Pain*, vol. 44, no. 3, pp. 301–310, 1991.

[87] D. A. Simone and J. Ochoa, "Early and late effects of prolonged topical capsaicin on cutaneous sensibility and neurogenic vasodilatation in humans," *Pain*, vol. 47, no. 3, pp. 285–294, 1991.

[88] M. Nolano, D. A. Simone, G. Wendelschafer-Crabb, T. Johnson, E. Hazen, and W. R. Kennedy, "Topical capsaicin in humans: parallel loss of epidermal nerve fibers and pain sensation," *Pain*, vol. 81, no. 1-2, pp. 135–145, 1999.

[89] N. Khalili, G. Wendelschafer-Crabb, W. R. Kennedy, and D. A. Simone, "Influence of thermode size for detecting heat pain dysfunction in a capsaicin model of epidermal nerve fiber loss," *Pain*, vol. 91, no. 3, pp. 241–250, 2001.

[90] A. B. Malmberg, A. P. Mizisin, N. A. Calcutt, T. von Stein, W. R. Robbins, and K. R. Bley, "Reduced heat sensitivity and epidermal nerve fiber immunostaining following single applications of a high-concentration capsaicin patch," *Pain*, vol. 111, no. 3, pp. 360–367, 2004.

[91] M. Polydefkis, P. Hauer, S. Sheth, M. Sirdofsky, J. W. Griffin, and J. C. McArthur, "The time course of epidermal nerve fibre regeneration: studies in normal controls and in people with diabetes, with and without neuropathy," *Brain*, vol. 127, no. 7, pp. 1606–1615, 2004.

[92] H. E. Torebjörk and R. G. Hallin, "Responses in human A and C fibres to repeated electrical intradermal stimulation," *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 37, pp. 653–664, 1974.

[93] H. J. Braune, "Testing of the refractory period in sensory nerve fibres is the most sensitive method to assess beginning polyneuropathy in diabetics," *Electromyography and Clinical Neurophysiology*, vol. 39, no. 6, pp. 355–359, 1999.

[94] K. Ørstavik, B. Namer, R. Schmidt et al., "Abnormal function of C-fibers in patients with diabetic neuropathy," *Journal of Neuroscience*, vol. 26, no. 44, pp. 11287–11294, 2006.

[95] R. de Col, K. Messlinger, and R. W. Carr, "Conduction velocity is regulated by sodium channel inactivation in unmyelinated axons innervating the rat cranial meninges," *Journal of Physiology*, vol. 586, no. 4, pp. 1089–1103, 2008.

[96] R. de Col, K. Messlinger, and R. W. Carr, "Repetitive activity slows axonal conduction velocity and concomitantly increases mechanical activation threshold in single axons of the rat cranial dura," *The Journal of Physiology*, vol. 590, pp. 725–736, 2012.

[97] Z. R. Zhu, X. W. Tang, W. T. Wang et al., "Conduction failures in rabbit saphenous nerve unmyelinated fibers," *NeuroSignals*, vol. 17, no. 3, pp. 181–195, 2009.

[98] A. George, J. Serra, X. Navarro, and H. Bostock, "Velocity recovery cycles of single C fibres innervating rat skin," *Journal of Physiology*, vol. 578, no. 1, pp. 213–232, 2007.

[99] O. Obreja, M. Ringkamp, B. Namer et al., "Patterns of activity-dependent conduction velocity changes differentiate classes of unmyelinated mechano-insensitive afferents including cold nociceptors, in pig and in human," *Pain*, vol. 148, no. 1, pp. 59–69, 2010.

[100] S. N. Lawson, "Phenotype and function of somatic primary afferent nociceptive neurones with C-, A$\delta$- or A$\alpha$/$\beta$-fibres," *Experimental Physiology*, vol. 87, no. 2, pp. 239–244, 2002.

[101] L. Djouhri and S. N. Lawson, "A$\beta$-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals," *Brain Research Reviews*, vol. 46, no. 2, pp. 131–145, 2004.