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ORIGINAL RESEARCH

Changes in functional properties of A-type but not C-type sensory neurons in vivo in a rat model of peripheral neuropathy

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Background: The aim of this study was to compare primary sensory neurons in controls and in an animal neuropathic pain model in order to understand which types of neurons undergo changes associated with peripheral neuropathy. On the basis of intracellular recordings in vivo from somata, L4 sensory dorsal root ganglion neurons were categorized according to action potential configuration, conduction velocity, and receptive field properties to mechanical stimuli.

Methods: Intracellular recordings were made from functionally identified dorsal root ganglion neurons in vivo in the Mosconi and Kruger animal model of peripheral neuropathic pain.

Results: In this peripheral neuropathy model, a specific population of Aβ-fiber low threshold mechanoreceptor neurons, which respond normally to innocuous mechanical stimuli, exhibited differences in action potential configuration and conduction velocity when compared with control animals. No abnormal conduction velocity, action potential shapes, or tactile sensitivity of C-fiber neurons were encountered.

Conclusion: This study provides evidence for defining a potential role of Aβ-fiber low threshold mechanoreceptor neurons that might contribute to peripheral neuropathic pain.

Keywords: peripheral neuropathy, neuropathic pain, primary sensory neuron, dorsal root ganglion, action potential configuration, animal model, in vivo recording

Introduction

Neuropathic pain is initiated or caused by a primary lesion or dysfunction in the nervous system[1,2] and includes postherpetic neuralgia, trigeminal neuralgia, diabetic neuropathy, spinal cord injury, cancer and chemotherapy, and stroke, as well as some degenerative neurological diseases.[3] In contrast to nociceptive pain, neuropathic pain is described as spontaneous burning pain with accompanying hyperalgesia and allodynia.[4] Tactile allodynia is a common stimulus-evoked response and is defined as pain resulting from a light touch that ordinarily does not elicit a painful response.

It has become evident that peripheral neuropathic pain is characterized by membrane ectopic activity that is thought to be generated in both damaged as well as neighboring intact/surviving fibers of primary sensory neurons.[5] Previous studies have shown that ectopic activity may arise from the dorsal root ganglion soma, along the axon, and in the peripheral nerve terminals,[6–11] and prolonged responses to sensory inputs of dorsal horn neurons in neuropathic rats are reduced by local anesthetic application to the peripheral sensory nerve.[12] These abnormal activities of peripheral neurons are suggested to play a role as a pain signal and as an inducer of central sensitization observed in animal models of peripheral neuropathy.[7,13–15]

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However, it is still not clear which functional subgroup of afferent neurons is involved in altering nociceptive scores in various animal models of peripheral neuropathic pain, and particularly whether or not nociceptors are involved. The major concepts or hypotheses in this regard are clearly described in a recent review by Devor.[5] One theory is the "excitable nociceptor hypothesis", which is based on a reduced response threshold in nociceptive afferents. Another hypothesis is that ectopic activity in low threshold mechanoreceptor afferents is abnormally "amplified" in the spinal cord.[16] Both hypotheses embrace the observation that C or Aβ fibers carry the ectopic activity in models of peripheral neuropathy. However, so far, most of the previous studies have been based on axotomized neurons in vitro that were anatomically and/or only partially functionally identified. Identifying axotomized dorsal root ganglion neurons as nociceptive or non-nociceptive is questionable because they are disconnected from their sensory receptors.

In our earlier studies using the Mosconi and Kruger rat model of neuropathic pain,[17] in which a polyethylene cuff is placed around a sciatic nerve unilaterally, we observed hypersensitivity to tactile stimuli, as assessed in the von Frey test; this is generally considered to be the animal equivalent of "allodynia" in humans.[18] Extracellular electrophysiological recordings made in this model showed an elevated discharge of wide dynamic range dorsal horn neurons in response to both noxious and innocuous mechanical stimulation of peripheral cutaneous receptive fields.[19,20] Further, in acutely spinalized animals, the hyperactivity of dorsal horn neurons was blocked by application of lidocaine to the sciatic nerve.[20] Together, this evidence suggests that increased wide dynamic range neuron discharge may be maintained by increased or exaggerated input from primary afferents that can be directly stimulated from peripheral cutaneous receptive fields or are spontaneously active. Therefore, to understand the possible contribution of primary sensory neurons to tactile hypersensitivity in this model, we recorded intracellularly from dorsal root ganglion somata using in vivo electrophysiological experiments, and fully characterized each neuron on the basis of several parameters, including the configuration of the action potential, the conduction velocity of the axon, and activation of the respective sensory receptive field. Comparisons were then made with the same properties of dorsal root ganglion neurons recorded from naive control animals.

We report here that low threshold mechanoreceptor neurons associated with Aβ fibers in particular undergo changes in functional properties and thus might play a role as an essential trigger of tactile hypersensitivity in the Mosconi and Kruger model and possibly in tactile allodynia after peripheral nerve injury; small neurons and high threshold large neurons exhibited either no change or only minor changes in these properties.

Materials and methods

All experimental procedures conformed to the Guide to the Care and Use of Laboratory Animals, Volumes 1 and 2, of the Canadian Council on Animal Care, and all protocols were approved by the McMaster University Animal Review Ethics Board. At the end of the acute electrophysiological experiment, the animal was euthanized without recovery by an anesthetic overdose.

Experimental animals and neuropathic surgery

Young male Sprague-Dawley rats (obtained from Charles River Inc, St Constant, QC, Canada) weighing 170–200 g were used. The animals were divided into two groups, ie, naive control and neuropathic model groups. A peripheral neuropathy was induced according to a method previously described in detail.[17,19,21] Under anesthesia with a mixture of ketamine 5 mg/100 g (Ketamine®; Bimeda-MTC Animal Health Inc, Cambridge, ON, Canada), xylazine (Rompun®; 0.5 mg/100 g; Bayer HealthCare, Toronto, ON, Canada), and acepromazine (Atravet®; 0.1 mg/100 g; Ayerst Veterinary Laboratories, Guelph, ON, Canada) given intraperitoneally, the right sciatic nerve was exposed at the mid-thigh level. Two cuffs of 0.5 mm polyethylene tubing (Intramedic PE-90, Fisher Scientific Ltd, Whitby, Ontario, Canada) were inserted around the exposed nerve about 1 mm apart. The wound was then sutured in two layers, ie, muscle and skin. Antibiotic ointment (Furacin®; nitrofurazone 0.2%; Vetoquinol N-A Inc, Lavaltrie, QC, Canada) was applied over the wound, and 0.01 mL/100 g of injectable antibacterial solution (Bayer HealthCare) was injected subcutaneously. Animals were given 1 mL of saline subcutaneously and an ocular lubricant, and placed under a heating lamp until they recovered from the anesthetic, and were then returned to their home cages.

von Frey test of paw withdrawal threshold

In all cases, the von Frey test was run on the same day as the recording day before the rats were anesthetized for the acute electrophysiological experiment. This was done to confirm that each animal had developed the tactile hypersensitivity that characterizes this model. To quantify mechanical sensitivity of the foot, brisk foot withdrawal in response to normally innocuous mechanical stimuli was measured as

described previously.[22] The rats were placed in a transparent Plexiglas box with a clear Plexiglas floor, containing 0.5 cm diameter holes spaced 1.5 cm apart to allowed full access to the paws.[12,19,21] The animals were allowed to habituate to the box for approximately 15 minutes, when cage exploration and major grooming activities had ceased.

von Frey filaments (Stoelting Co, Wood Dale, IL) were applied to the plantar surface of the hind paw to determine the withdrawal threshold. A von Frey filament was applied five times (for 3–4 seconds each, at 3-second intervals) to a different spot on each hind paw. For each rat, these filaments were applied in ascending order until a clear withdrawal response was observed. When this occurred, the next lightest filament was applied following the same procedure. A 50% withdrawal response threshold was derived according to responses to this testing regimen,[23] using the up-down method of Dixon.[24] Brisk foot withdrawal in response to these mechanical stimuli was interpreted as indicating mechanical hypersensitivity.

Intracellular recording in vivo

Details of the surgical preparation and intracellular recording approaches have been reported previously.[25,26] In brief, the rat was initially anesthetized with the anesthetic mixture described above. The right jugular vein was cannulated for intravenous infusion of drugs. The rat was then fixed in a stereotaxic frame and the vertebral column rigidly clamped at the L2 and L6 vertebral levels. The right femur was fixed by a customized clamp onto the stereotaxic frame to minimize movement of the dorsal root ganglion during mechanical searching for receptive fields on the leg. The L4 dorsal root ganglion was selected for study because it contains large numbers of hind leg afferent somata. A laminectomy was performed to expose the ipsilateral L4 dorsal root ganglion. The L4 dorsal root was sectioned close to the spinal cord and placed on a bipolar electrode (FHC, Bowdoinham, ME) used for stimulation purposes. The exposed spinal cord and dorsal root ganglion were covered with warm paraffin oil at 37°C to prevent drying. Rectal temperature was maintained at about 37°C using a temperature-controlled infrared heating lamp.

For recording, each rat was anesthetized at a surgical level with sodium pentobarbital (20 mg/kg; Ceva Sante Animal, Libourne, France). The rat was mechanically ventilated via a tracheal cannula using a Harvard Ventilator (Model 683, Harvard apparatus, Quebec, Canada). The ventilation parameters were adjusted so that the end-tidal CO_2 concentration was maintained at around 40–50 mmHg, as measured using a CapStar-100 end-tidal CO_2 analyzer (CWE, Ardmore, PA). Immediately before the start of recording, an initial 1 mg/kg dose of pancuronium (Sandoz, Boucherville, QC, Canada) was given to eliminate muscle tone. The effects of pancuronium were allowed to wear off periodically to confirm a surgical level of anesthesia; this was monitored by observing pupil diameter and any response to noxious pinch of a forepaw. Supplements of pentobarbital and pancuronium were given at a dose of 1/3 the original dose approximately each hour via the jugular cannula.

Intracellular recordings from somata in the exposed dorsal root ganglion were made with borosilicate glass micropipettes (1.2 mm outside diameter, 0.68 mm inside diameter; Harvard Apparatus, Holliston MA). The electrodes were pulled using a Brown-Flaming puller (model p-87; Sutter Instrument Co, Novota, CA). These electrodes were filled with 3 M KCl (DC resistance 50–70 mΩ). Signals were recorded with a Multiclamp 700B amplifier (Molecular Devices, Union City CA) and digitized online via Digidata 1322A interface (Molecular Devices) with pClamp 9.2 software (Molecular Devices). The microelectrode was advanced using an EXFO IW-800 micromanipulator (EXFO, Montreal, QC, Canada) in 2 μm steps until a hyperpolarization of at least 40 mV suddenly appeared. Once a stable membrane potential had been confirmed, a single stimulus was applied to the dorsal roots to provoke an action potential; with the aid of the protocol editor function in pClamp 9.2 software, a somatic action potential was evoked by stimulation with a single rectangular voltage pulse.

Dorsal root ganglion neuron classification

The neurons were divided into three groups on the basis of dorsal root conduction velocity. The conduction velocity range was ≤0.8 m/sec for C-fiber neurons, 1.5–6.5 m/sec for Aδ-fiber neurons, and >6.5 m/sec for Aβ-fiber neurons, as defined elsewhere.[25–29]

The sensory receptive properties of each dorsal root ganglion neuron were examined using hand-held mechanical stimulators and classified as previously described.[25,26,29,30] Differentiation between high threshold mechanoreceptor neurons and low threshold mechanoreceptor neurons was based on their sensory properties identified during receptive field searching. High threshold mechanoreceptor neurons responded to noxious stimuli including noxious pressure, pinch, probing with fine forceps, a sharp needle, coarse-toothed forceps, or coarse flat forceps, whereas low threshold mechanoreceptor neurons responded to innocuous stimuli such as a moving brush, light pressure with a blunt object, light manual tap, or vibration. Neurons that did not respond

to any of the non-noxious or noxious mechanical stimuli were classified as unresponsive, as previously described.[29]

In addition to the threshold of activation, the rate of adaption and tissue location of the receptive field were other major factors used to classify Aβ-fiber low threshold mechanoreceptor neurons further as guard/field hair neurons, glabrous skin neurons, Pacinian neurons, slowly adapting neurons, and muscle spindle neurons. Guard/field hair neurons were rapidly adapting cutaneous neurons. Glabrous and Pacinian neurons were both rapidly adapting non-hair neurons, and were named rapidly adapting neurons. Slowly adapting neurons were slowly adapting cutaneous neurons. Muscle spindle neurons were slowly adapting neurons with deep subcutaneous receptive fields activated by deep tissue manipulation of the muscle belly but not by cutaneous stimulation.

Action potential configuration

The first evoked action potential in each neuron was used to determine any differences in configuration between control and neuropathic animals. Criteria for acceptance of neurons in the analysis included a stable Vm more negative than about 40 mV, with a somatic spike evoked by dorsal root stimulation that was >40 mV.

Variables in action potential configuration included resting membrane potential (Vm), action potential amplitude, action potential duration at base, action potential rise time, action potential fall time, maximum action potential rising rate, maximum action potential falling rate, afterhyperpolarization amplitude, and afterhyperpolarization duration to 50% recovery (AHP_{50}).

Conduction velocity

The distance from the stimulation site (cathode) to the recording site (center of the dorsal root ganglion) was measured at the end of the experiment to determine the conduction distance. This was then used to calculate the conduction velocity of the dorsal root axon associated with each neuron.

Mechanical sensitivity test during intracellular recording

The mechanical sensitivity of high threshold mechanoreceptor neurons was determined individually using calibrated von Frey filaments as described previously.[30–32] Briefly, after functional classification of a neuron using the hand-held mechanical stimulators, von Frey filaments were applied to the identified receptive field, and the mechanical activation threshold of each neuron was expressed as the minimum force (g) necessary to evoke impulses on the most sensitive spot on the skin. The mechanical forces exerted with the calibrated von Frey filaments used in this study were a set of von Frey filaments exerting pressures of 0.008, 0.02, 0.04, 0.07, 0.16, 0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10, 15, 26, 60, 100, 180 and 300 g; tip diameters ranged from 1.65 mm to 6.65 mm.

Statistical analysis

Normality of electrophysiological data was done with the D'Agostino and Pearson omnibus test. Wherever appropriate, the Student's *t*-test or the Mann-Whitney *U*-test was used for comparisons between neuropathic and control animals in various neuronal subtypes and for various parameters. All statistical tests and graphing was done using Prism4 software (Graphpad, La Jolla, CA). $P < 0.05$ was considered to indicate a statistically significant difference, as shown in the graphs.

Results

Withdrawal threshold in the von Frey test

Behavioral tests of tactile hypersensitivity were made from a total of 124 rats (60 control and 64 neuropathic rats). Stimulation of the plantar surface of the hind paw with von Frey filaments evoked a withdrawal response in control animals, with hairs exerting pressures of 10–100 g. Three weeks after cuff ligation of the sciatic nerve on the right side, model rats fully developed behavioral signs of neuropathic pain on the affected hind limb; filaments to which the control animals showed no withdrawal response, ie, 0.001–6.0 g, in neuropathic animals evoked a clear withdrawal of the nerve-injured hind limb. Furthermore, withdrawal was greatly exaggerated in amplitude and duration, and was frequently accompanied by licking of the paw. Withdrawal thresholds were 14.44 ± 0.221 g in control animals (n = 60) and 4.52 ± 0.69 g in neuropathic animals (n = 64); comparison of the data indicated $P < 0.0001$.

Electrophysiological recording

Intrasomal recordings in these rats were made from a total of 399 L4 dorsal root ganglion neurons (175 neurons in control animals and 224 neurons in neuropathic animals). In control rats, these included 33 C-fiber (21 high threshold mechanoreceptor, eight low threshold mechanoreceptor, and four unresponsive units), 22 Aδ-fiber (11 high threshold mechanoreceptor, seven low threshold mechanoreceptor, four unresponsive units), 120 Aβ-fiber (26 high threshold mechanoreceptor, 86 low threshold mechanoreceptor, and eight unresponsive) neurons.

In neuropathic animals, these included 40 C-fiber (28 high threshold mechanoreceptor, eight low threshold mechanoreceptor, four unresponsive units), 20 Aδ-fiber (seven high threshold mechanoreceptor, nine low threshold mechanoreceptor, four unresponsive units), and 164 Aβ-fiber (39 high threshold mechanoreceptor, 116 low threshold mechanoreceptor and nine unresponsive units) neurons. All neurons included in these results met the inclusion criteria described above. Examples of action potentials recorded from individual neuron types are illustrated in Figure 1. With respect to Aβ-fiber low threshold mechanoreceptor neurons, 20 guard/field hair neurons were recorded in control and 25 in neuropathic rats. Similarly, 20 rapidly adapting neurons were recorded in control and 24 in neuropathic rats, 10 slowly adapting neurons were recorded in control rats and 14 in neuropathic rats. Thirty-six muscle spindle neurons were recorded in control rats and 53 in neuropathic rats.

Action potential configuration

The various action potential configuration parameters of corresponding subclasses in each conduction velocity group were compared between control and neuropathic rats. All data are shown in Table 1 and the scatter plots of Figures 2, 3, and 4.

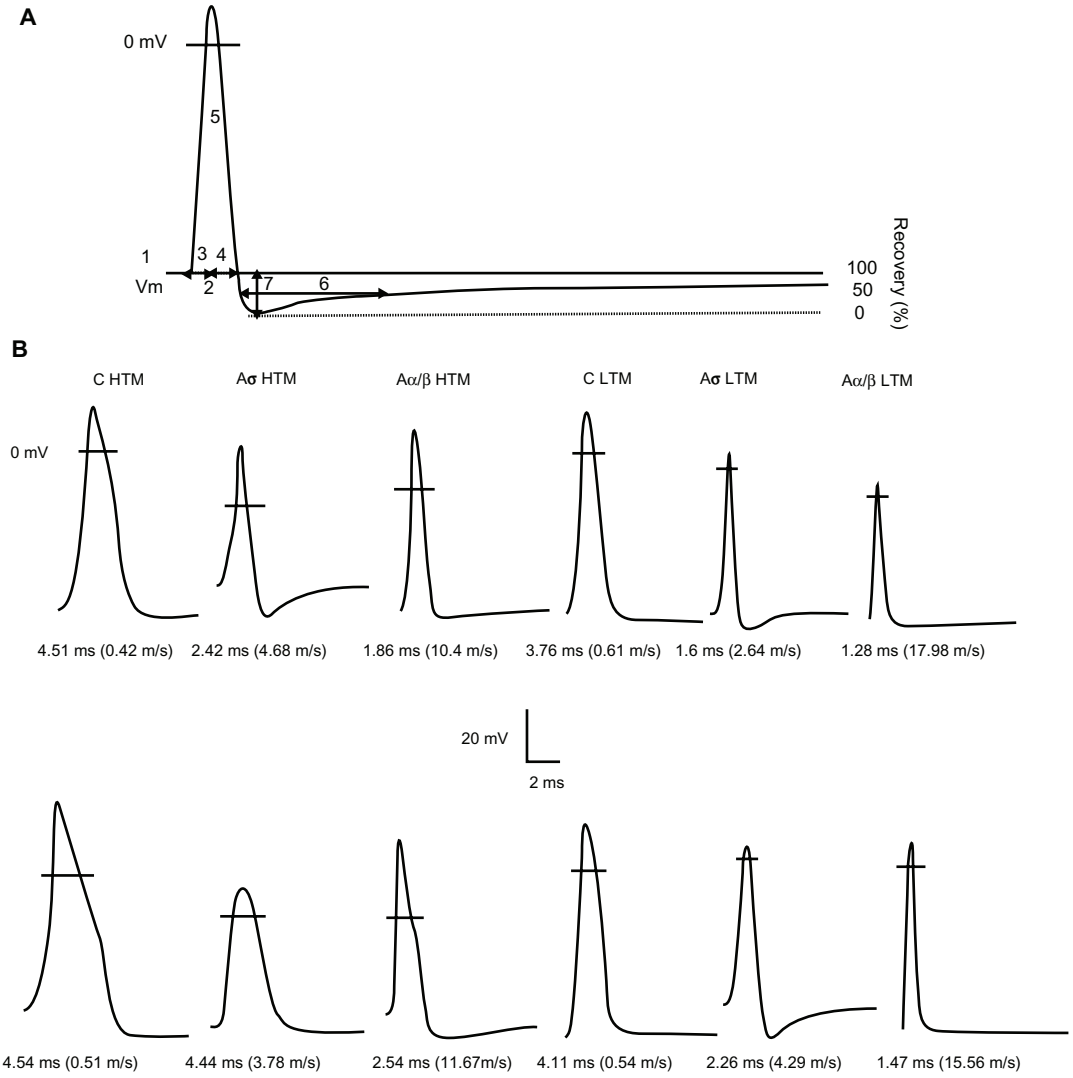

Figure 1 Examples of APs recorded from mechanoreceptive neurons. (**A**) Representative intracellular somatic action potential of an A-fiber neuron evoked by electrical stimulation of the dorsal root showing the electrophysiological parameters measured, including: **1**, resting membrane potential; **2**, action potential duration at base; **3**, action potential rise time; **4**, action potential fall time; **5**, action potential amplitude; **6**, AHP duration to 50% recovery; **7**, and afterhyperpolarization amplitude below Vm. In addition, maximum rising and falling rates, (dV/d*t*) max, were measured from the differential trace of the action potential. (**B**) Somatic action potentials evoked by dorsal root stimulation and recorded intracellularly from 12 mechanoreceptive neurons selected to represent the mean action potential duration values for each of the different groups of neurons in control (upper) and neuropathic (lower) animals. The action potential duration and conduction velocity for each neuron are given below each record. The horizontal lines across the action potentials indicate zero membrane potential.

Abbreviations: HTM, high threshold mechanoreceptive neurons; LTM, low threshold mechanoreceptive neurons.

Table 1 Comparison of properties of high-threshold mechanoreceptive and low-threshold mechanoreceptive dorsal root ganglion neurons between control and neuropathic rats

CV range	Receptor class	Model	n	CV (m/s)	Vm (–mV)	APA (mV)	APdB (ms)	APRT (ms)	APFT (ms)	MRR (mV/ms)	MFR (mV/ms)	AHPA (mV)	AHP_{50} (ms)
C	HTM	Control	21	0.59 ± 0.040	62.12 ± 2.130	83.80 ± 2.092	3.76 ± 0.247	1.47 ± 0.092	2.27 ± 0.181	146.5 ± 8.587	60.89 ± 3.390	8.73 ± 0.877	11.14 ± 1.888
		NeP	28	0.60 ± 0.036	60.35 ± 1.626	77.58 ± 2.725	3.99 ± 0.296	1.41 ± 0.111	2.40 ± 0.210	151.5 ± 10.81	58.04 ± 3.039	9.68 ± 0.771	10.95 ± 1.614
				$P = 0.8355$	$P = 0.5061$	$P = 0.0934$	$P = 0.5741$	$P = 0.6801$	$P = 0.6515$	$P = 0.7354$	$P = 0.5356$	$P = 0.4217$	$P = 0.9417$
	LTM	Control	8	0.47 ± 0.058	72.79 ± 1.866	70.89 ± 3.526	3.37 ± 0.273	1.28 ± 0.096	2.16 ± 0.177	161.5 ± 18.85	73.94 ± 1.437	1.40 ± 0.437	10.05 ± 1.054
		NeP	8	0.50 ± 0.033	71.62 ± 1.817	71.10 ± 2.91	3.19 ± 0.267	1.30 ± 0.052	2.26 ± 0.236	173.4 ± 10.91	71.27 ± 1.84	1.78 ± 0.347	9.68 ± 0.861
				$P = 0.6855$	$P = 0.6583$	$P = 0.9579$	$P = 0.6361$	$P = 0.8836$	$P = 0.7336$	$P = 0.5925$	$P = 0.2878$	$P = 0.5069$	$P = 0.7869$
	Unresponsive	Control	4	0.60 ± 0.054	59.67 ± 5.840	82.71 ± 4.650	3.74 ± 0.429	1.45 ± 0.246	2.18 ± 0.826	147.7 ± 22.08	68.29 ± 5.938	8.67 ± 3.346	11.56 ± 2278
		Nep	4	0.61 ± 0.094	59.59 ± 6.306	74.37 ± 7.491	3.95 ± 1.139	1.44 ± 0.334	2.10 ± 0.686	154.0 ± 43.60	59.51 ± 4.406	8.09 ± 1.659	11.86 ± 2.856
				$P = 0.9469$	$P = 0.9933$	$P = 0.3811$	$P = 0.8718$	$P = 0.9769$	$P = 0.9377$	$P = 0.9016$	$P = 0.2801$	$P = 0.8802$	$P = 0.9372$
Aσ	HTM	Control	11	4.32 ± 0.514	53.96 ± 3.875	74.70 ± 3.300	2.49 ± 0.289	1.03 ± 0.123	1.40 ± 0.204	193.5 ± 13.69	84.53 ± 12.50	9.51 ± 1.494	9.68 ± 2.241
		NeP	7	4.86 ± 0.505	53.86 ± 3.341	78.75 ± 3.829	3.87 ± 0.604	1.06 ± 0.075	2.66 ± 0.518	168.3 ± 23.21	62.09 ± 9.594	9.68 ± 2.564	8.32 ± 1.910
				$P = 0.4871$	$P = 0.9867$	$P = 0.4429$	$P = 0.0355$*	$P = 0.8692$	$P = 0.0180$*	$P = 0.3311$	$P = 0.2189$	$P = 0.9519$	$P = 0.6776$
	LTM	Control	7	5.17 ± 0.705	70.65 ± 4.122	66.92 ± 3.867	1.76 ± 0.155	0.77 ± 0.081	0.98 ± 0.098	202.8 ± 15.22	123.1 ± 7.946	5.72 ± 1.695	3.22 ± 0.628
		NeP	9	3.91 ± 0.500	62.23 ± 2.126	62.59 ± 2.602	2.51 ± 0.412	0.93 ± 0.092	1.58 ± 0.387	148.0 ± 13.37	89.84 ± 8.396	7.44 ± 1.463	4.14 ± 1.255
				$P = 0.1548$	$P = 0.0727$	$P = 0.3519$	$P = 0.1472$	$P = 0.2291$	$P = 0.2091$	$P = 0.170$*	$P = 0.0138$*	$P = 0.4545$	$P = 0.5601$
	Unresponsive	Control	3	3.21 ± 1.084	54.16 ± 4.564	75.26 ± 5.250	1.98 ± 0.262	0.88 ± 0.15	1.14 ± 0.205	195.5 ± 17.24	76.23 ± 21.145	8.54 ± 3.340	12.87 ± 3.639
		NeP	3	4.14 ± 1.472	53.28 ± 3.183	79.11 ± 5.968	2.71 ± 0.619	1.17 ± 0.06	2.77 ± 0.758	163.1 ± 26.37	74.371 ± 10.165	10.34 ± 3.344	13.3 ± 2.652
				$P = 0.6365$	$P = 0.8704$	$P = 0.6449$	$P = 0.1616$	$P = 0.2445$	$P = 0.0834$	$P = 0.3204$	$P = 0.6313$	$P = 0.7617$	$P = 0.9266$
Aβ	HTM	Control	26	12.42 ± 0.548	62.80 ± 2.280	75.18 ± 2.116	1.73 ± 0.061	0.71 ± 0.041	1.02 ± 0.032	262.9 ± 14.87	141.9 ± 9.230	9.39 ± 0.614	10.69 ± 1.551
		NeP	39	11.38 ± 0.504	63.21 ± 1.783	75.47 ± 1.674	2.45 ± 0.146	0.86 ± 0.054	1.58 ± 0.132	211.5 ± 11.07	94.47 ± 9.214	6.57 ± 0.765	8.98 ± 0.931
				$P = 0.1794$	$P = 0.8863$	$P = 0.9127$	$P = 0.0003$***	$P = 0.0492$*	$P = 0.0012$**	$P = 0.0281$*	$P = 0.0118$*	$P = 0.0101$*	$P = 0.3184$
	LTM (SKIN)	Control	50	17.02 ± 0.469	68.63 ± 1.100	63.09 ± 1.508	1.25 ± 0.038	0.49 ± 0.019	0.76 ± 0.028	262.4 ± 8.735	142.7 ± 4.855	5.51 ± 0.493	5.42 ± 0.834
		NeP	63	14.70 ± 0.548	65.63 ± 0.962	63.16 ± 1.292	1.48 ± 0.065	0.57 ± 0 019	0.90 ± 0.050	226.2 ± 7.947	131.7 ± 5.882	4.89 ± 0.365	5.65 ± 0.743
				$P = 0.0023$**	$P = 0.0423$*	$P = 0.9704$	$P = 0.0046$*	$P = 0.0027$**	$P = 0.0222$*	$P = 0.0027$**	$P = 0.1641$	$P = 0.3045$	$P = 0.8398$
	LT (MS)	Control	36	18.76 ± 0.638	66.47 ± 1.704	57.83 ± 0.929	0.96 ± 0.032	0.43 ± 0.017	0.53 ± 0.022	258.4 ± 12.44	186.0 ± 8.476	6.73 ± 0.678	1.58 ± 0.114
		NeP	53	16.69 ± 0.599	62.60 ± 1.055	56.63 ± 1.107	1.10 ± 0.045	0.49 ± 0.016	0.62 ± 0.033	225.9 ± 8.019	155.0 ± 5.126	7.07 ± 0.581	2.20 ± 0.146
				$P = 0.0235$*	$P = 0.0442$*	$P = 0.4422$	$P = 0.0229$*	$P = 0.0152$*	$P = 0.0467$*	$P = 0.0239$*	$P = 0.0013$**	$P = 0.7089$	$P = 0.0030$**
	Unresponsive	Control	8	12.67 ± 0.831	63.48 ± 2.774	81.95 ± 2.107	1.63 ± 0.062	0.63 ± 0.021	1.03 ± 0.048	258.4 ± 13.18	133.5 ± 7.025	8.34 ± 0.719	10.26 ± 3.545
		NeP	9	11.15 ± 1.088	61.03 ± 3.482	79.13 ± 3.438	2.29 ± 0.251	0.77 ± 0.093	1.53 ± 0.260	221.4 ± 23.38	104.8 ± 15.18	5.23 ± 1.207	9.74 ± 1.805
				$P = 0.2922$	$P = 0.5966$	$P = 0.5083$	$P = 0.0297$*	$P = 0.1891$	$P = 0.0948$	$P = 0.2023$	$P = 0.1270$	$P = 0.0487$*	$P = 0.8933$

Notes: Statistical tests for each variable were made in all subgroups comparing neurons in control and neuropathic rats. The means ± standard deviations of variables measured are given. The value of *P* (mean-Whitney) is shown below each section. The asterisks show the level of significance of the difference between neurons in control and neuropathic animals: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Abbreviations: n, the number of neurons in each group; CV, conduction velocity; Vm, resting membrane potential; APA, action potential amplitude; APdB, action potential duration at base; APRT, action potential rise time; APFT, action potential fall time; MRR, maximum rising rate; MFR, maximum falling rate; AHPA, afterhyperpolarization amplitude; AHP_{50}, afterhyperpolarization duration at 50% recovery; HTM, high threshold mechanoreceptive neurons; LTM, low threshold mechanoreceptive neurons; UN, unresponsive neurons; MS, muscle spindle neurons.

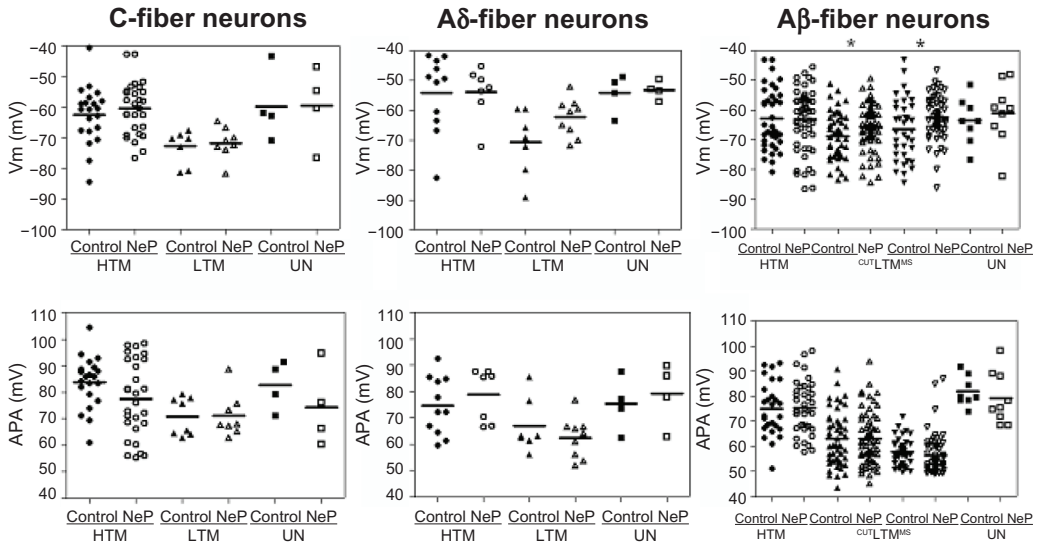

Figure 2 Comparison of action potential resting membrane potential and amplitude of dorsal root ganglion neurons between control and neuropathic rats. Scatter plots show the distribution of the variables with the median (horizontal line) superimposed in each case.

Notes: Asterisks above the graph indicate the significant differences between control and neuropathic animals: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. The absence of an asterisk indicates lack of a statistically significant difference.

Abbreviations: HTM, high threshold mechanoreceptor neurons; LTM, low threshold mechanoreceptor neurons; UN, unresponsive neurons; CUT, Aβ LTM including guard/field hair neurons, rapidly adapting neurons and slowly adapting neurons; MS, Aβ LTM muscle spindle neurons.

Resting membrane potential

Values of Vm for all neurons included in this study are given in Table 1. Vm of all subtypes of C-fiber neurons was similar in control and neuropathic rats: this was the case for high threshold mechanoreceptor neurons, low threshold mechanoreceptor neurons, and unresponsive neurons. In all subtypes of Aδ-fiber neurons and Aβ-fiber high threshold mechanoreceptor neurons and unresponsive neurons, Vm was also similar between control and neuropathic rats. However, Vm in Aβ-fiber low threshold mechanoreceptor neurons in neuropathic rats was more depolarized than that in control rats, including both cutaneous and deep neurons, ie, cutaneous neurons (control, n = 50 versus neuropathic, n = 63; $P = 0.0423$) and muscle spindle neurons (control, n = 36 versus neuropathic, n = 53; $P = 0.0442$). These data are illustrated in Figure 2A.

Action potential amplitude

There were no significant differences in action potential amplitude between the control and neuropathic rats for any subtype of neurons in any conduction velocity group, as illustrated in Table 1. The data are shown in Figure 2B.

Action potential duration at base

The action potential duration at base differed between control and neuropathic rats in some neuron types, Aβ- and Aδ-fiber neurons in particular, as shown in Table 1. Thus, none of the subgroups of C-fiber neurons exhibited a difference between control and neuropathic rats. Of the Aδ-fiber neurons, only high threshold mechanoreceptor neurons exhibited a difference between control and neuropathic rats (control, n = 11 neurons versus neuropathic, n = 7 neurons; $P = 0.0355$). No difference was seen between control and neuropathic rats for low threshold mechanoreceptor or unresponsive neurons.

In marked contrast to C-fiber neurons, all Aβ-fiber neuron groups were different. Aβ-fiber neurons exhibited a longer action potential duration at base in neuropathic animals compared with controls, including Aβ-fiber high threshold mechanoreceptor neurons (control, n = 26 neurons versus neuropathic, n = 39 neurons; $P = 0.0003$), low threshold mechanoreceptor cutaneous neurons (control, n = 50 neurons versus neuropathic, n = 63 neurons; $P = 0.0046$), low threshold mechanoreceptor muscle spindle neurons (control, n = 36 neurons versus neuropathic, n = 53 neurons; $P = 0.0229$), and unresponsive neurons (control, n = 8 neurons versus neuropathic, n = 9 neurons; $P = 0.0297$). The data are shown in Figure 3A.

Action potential rise time

Action potential rise time did not differ between control and neuropathic rats in either C-fiber neurons or Aδ-fiber neurons. However, a longer action potential rise time was observed in the subgroups of Aβ-fiber neurons, with the exception of the unresponsive neurons. The data are presented in Table 1.

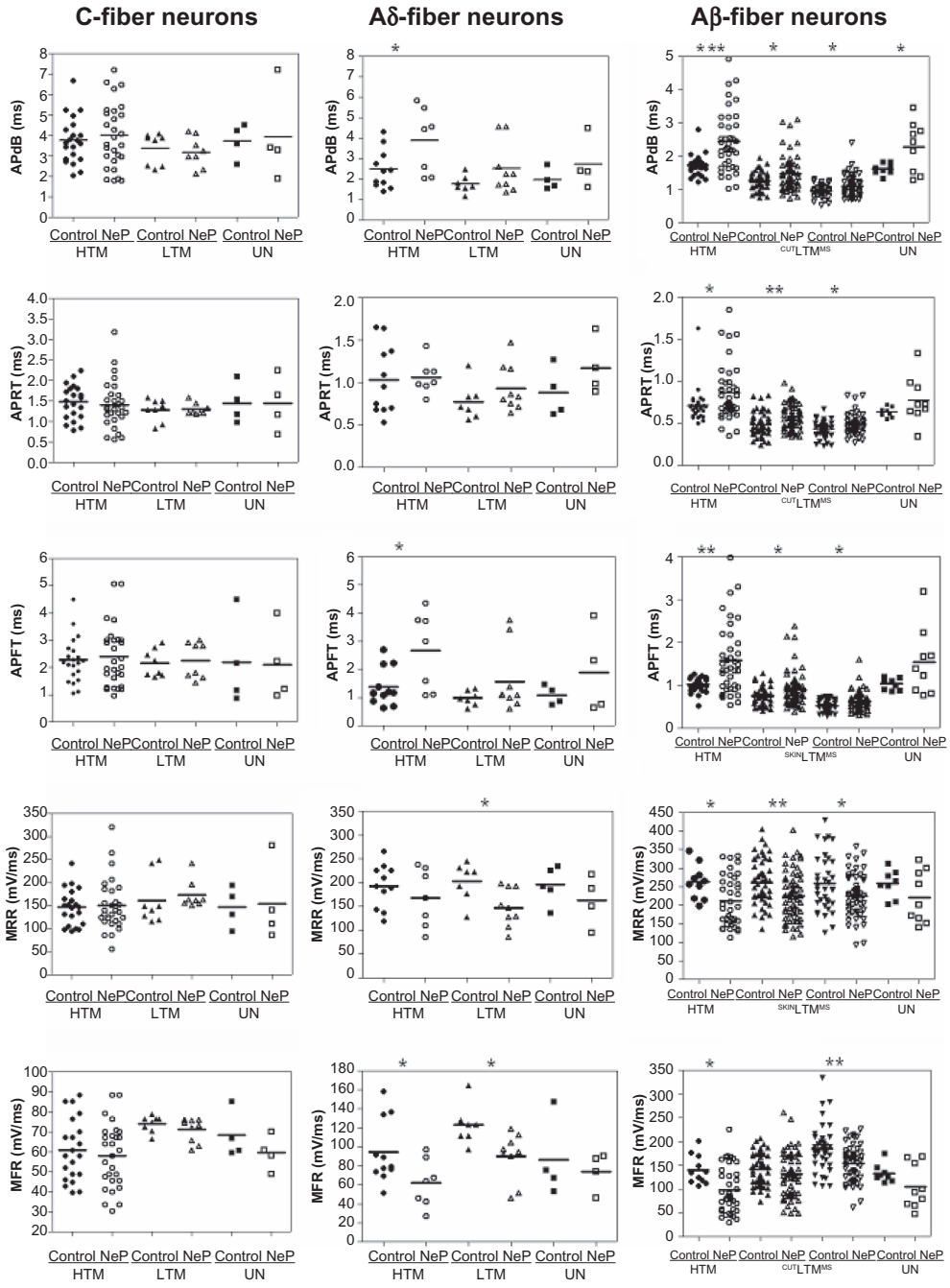

Figure 3 Comparison of action potential dynamic parameters of dorsal root ganglion neurons between control and neuropathic rats. Scatter plots show the distribution of the variables with the median (horizontal line) superimposed in each case.

Notes: Asterisks above the graph indicate the significant differences between control and neuropathic animals: *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$. The absence of an asterisk indicates the lack of a statistically significant difference.

Abbreviations: APdB, action potential duration at base; APRT, action potential rise time; APFT, action potential fall time; MRR, maximum AP rising rate; MFR, maximum AP falling rate; HTM, high threshold mechanoreceptor neurons; LTM, low threshold mechanoreceptor neurons; UN, unresponsive neurons.

Types of Aβ-fiber neurons that exhibited a statistically significant difference between control and neuropathic rats included high threshold mechanoreceptor neurons (control, n = 26 versus neuropathic, n = 39; $P = 0.0492$), low threshold mechanoreceptor cutaneous neurons (control, n = 50 versus neuropathic, n = 63; $P = 0.0027$), and low threshold mechanoreceptor muscle spindle neurons (control, n = 36 versus neuropathic, n = 53; $P = 0.0152$).

Action potential fall time

The action potential fall time also showed a differential effect on different neuron types between control and

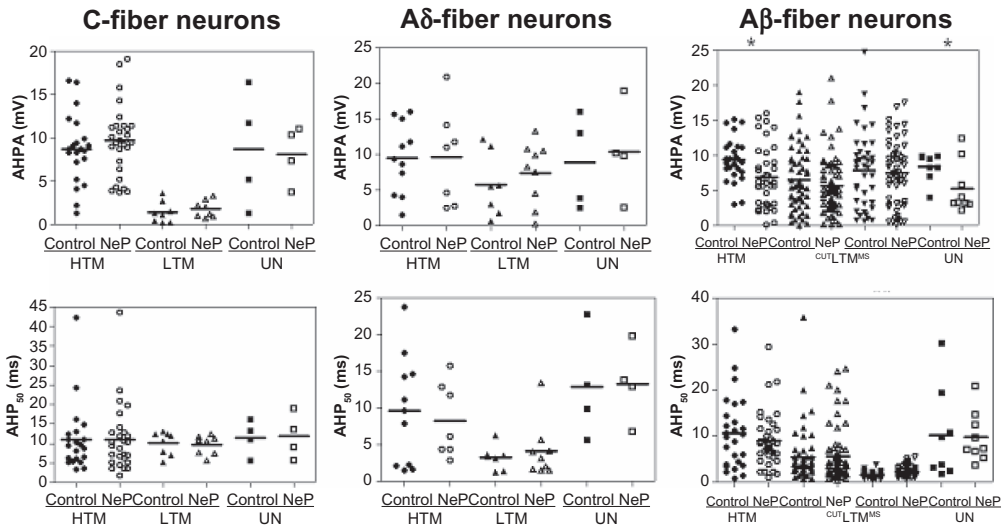

Figure 4 Comparison of AHP variables of DRG neurons between control and neuropathic rats. Scatter plots show the distribution of the variables with the median (horizontal line) superimposed in each case.

Notes: Asterisks above the graph indicate significant differences between control and neuropathic animals: $*P < 0.05$, $**P < 0.01$; $***P < 0.001$. The absence of an asterisk indicates the lack of a statistically significant difference.

Abbreviations: AHPA, afterhyperpolarization amplitude; AHP_{50}, afterhyperpolarization duration to 50% recovery; HTM, high threshold mechanoreceptor neurons; LTM, low threshold mechanoreceptor neurons; UN, unresponsive neurons.

neuropathic rats. The data are presented in Table 1. Thus, C-fiber neurons did not show a difference, whether high threshold mechanoreceptor neurons, low threshold mechanoreceptor neurons, or unresponsive neurons. In Aδ-fiber neurons, both high threshold mechanoreceptor and low threshold mechanoreceptor neurons exhibited longer action potential fall times in neuropathic than in control rats; high threshold mechanoreceptor (control, n = 11 versus neuropathic, n = 7; $P = 0.0180$) and low threshold mechanoreceptor (control, n = 7 versus neuropathic, n = 9; $P = 0.0356$). In contrast, unresponsive neurons were the same in both control and neuropathic rats. In Aβ-fiber neurons, all but the unresponsive neuron types displayed longer action potential fall times in neuropathic compared with control rats. Thus, longer action potential fall times were observed in neuropathic rats for high threshold mechanoreceptor neurons (control, n = 26 versus neuropathic, n = 39; $P = 0.0012$), low threshold mechanoreceptor cutaneous neurons (control, n = 50 versus neuropathic, n = 63; $P = 0.0222$), and low threshold mechanoreceptor muscle spindle neurons (control, n = 36 versus neuropathic, n = 53; $P = 0.0467$). These data are shown in Figure 3C.

Maximum rising rate

The action potential rising rate was slower in some but not all subtypes of neurons, as shown in Table 1. C-fiber neurons did not show a difference, whether high threshold mechanoreceptor neurons, low threshold mechanoreceptor neurons, or unresponsive neurons. Aδ-fiber low threshold mechanoreceptor neurons displayed a slower action potential rising rate in neuropathic rats compared with control rats (control, n = 7 versus neuropathic, n = 9; $P = 0.0337$). Neither high threshold mechanoreceptor neurons nor unresponsive neurons differed between neuropathic or control rats with respect to action potential rising rate. Of Aβ-fiber neurons, all showed a slower action potential rising rate in neuropathic rats compared with control rats, with the exception of the unresponsive neurons. Thus, a slower action potential rising rate was observed in high threshold mechanoreceptor neurons (control, n = 261 versus neuropathic, n = 39; $P = 0.0281$), low threshold mechanoreceptor cutaneous neurons (control, n = 50 versus neuropathic, n = 63; $P = 0.0027$), and low threshold mechanoreceptor muscle spindle neurons (control, n = 361 versus neuropathic, n = 53; $P = 0.0239$). The unresponsive neurons displayed a similar action potential rising rate in both groups of rats. The data are shown in Figure 3D.

Maximum falling rate

The maximum action potential falling rate was also slower in some neuron types in neuropathic rats compared with controls. Table 1 shows the data. As above, such changes were not observed in C-fiber neurons, including high threshold mechanoreceptor neurons, low threshold mechanoreceptor neurons, and unresponsive neurons. Aδ-fiber neurons showed a different distribution of slowing of maximum action potential falling rate in the neuron types compared with that seen earlier with the action potential rising rate. For example, low threshold

mechanoreceptor neurons displayed a slower maximum action potential falling rate in neuropathic rats compared with control rats (control, n = 7 versus neuropathic, n = 9; $P = 0.0138$). High threshold mechanoreceptor neurons also showed a slowing of maximum AP falling rate (control, n = 11 versus neuropathic, n = 7; $P = 0.0444$). However, unresponsive neurons did not show a difference in maximum action potential falling rate between rats. Aβ-neurons also displayed a neuron-type based grouping of differences in maximum action potential falling rate. The maximum action potential falling rate was different in high threshold mechanoreceptor neurons (control, n = 26 versus neuropathic, n = 39; $P = 0.0118$) and low threshold mechanoreceptor muscle spindle neurons (control, n = 36 versus neuropathic, n = 53; $P = 0.0013$), while there was no difference in maximum action potential falling rate in low threshold mechanoreceptor cutaneous neurons or in unresponsive neurons. The data are shown in Figure 3E.

AHP amplitude

A difference in AHP amplitude was seen only in two populations of Aβ-fiber neurons, comparing neuropathic versus control rats. These neuron types were Aβ-fiber high threshold mechanoreceptor neurons and unresponsive neurons. Thus, of the C-fiber neurons, none showed a difference between control and neuropathic animals, including the high threshold mechanoreceptor neurons, low threshold mechanoreceptor neurons, and unresponsive neurons. Similarly, of the neurons in the Aδ-fiber range, no subtypes showed a difference between control and neuropathic rats. In the Aβ-fiber range, AHP amplitude differed in high threshold mechanoreceptor neurons (control, n = 26 versus neuropathic, n = 39; $P = 0.0101$) and unresponsive neurons (control, n = 8 versus neuropathic, n = 9; $P = 0.0487$), but did not change in low threshold mechanoreceptor cutaneous or low threshold mechanoreceptor muscle spindle neurons. The data are shown in Figure 4.

AHP_{50} was different between control and neuropathic rats only in low threshold mechanoreceptor muscle spindle neurons. None of the C-fiber and Aδ-fiber neurons showed a difference between control and neuropathic animals, including the high threshold mechanoreceptor, low threshold mechanoreceptor, and unresponsive neurons. Of the neurons in the Aβ-fiber range, the AHP_{50} was longer only in the low threshold mechanoreceptor muscle spindle group (control, n = 36 versus neuropathic, n = 53; $P = 0.0030$). There was no difference between control and neuropathic animals in the other types of neurons in the Aβ-fiber range, including high threshold mechanoreceptor neurons, low threshold mechanoreceptor cutaneous neurons, and unresponsive neurons. The data are shown in Figure 4.

Conduction velocity

The conduction velocity was studied because it reflects properties of the axon rather than of the soma, as pertains to the data presented above. Figure 5 illustrates the distributions of conduction velocities for individual neurons in each neuron type in control and neuropathic rats. Comparison between these groups of rats for each type of C-fiber and Aδ-fiber neurons did not show a significant difference. However, in the Aβ-fiber range, low threshold mechanoreceptor neurons showed significantly slower conduction velocity in neuropathic animals.

Thus, in C-fiber neurons, the conduction velocity was 0.59 ± 0.040 m/sec (n = 21) and 0.60 ± 0.036 m/sec (n = 28) in control and neuropathic high threshold mechanoreceptor neurons, respectively ($P = 0.8355$). In low threshold mechanoreceptor neurons, the conduction velocity was 0.47 ± 0.058 m/sec (n = 8) and 0.50 ± 0.033 m/sec (n = 8) in control and neuropathic rats, respectively ($P = 0.6855$), and conduction velocity was 0.60 ± 0.054 m/sec (n = 4) and 0.61 ± 0.094 m/sec (n = 4) in control and neuropathic rats, respectively ($P = 0.9469$).

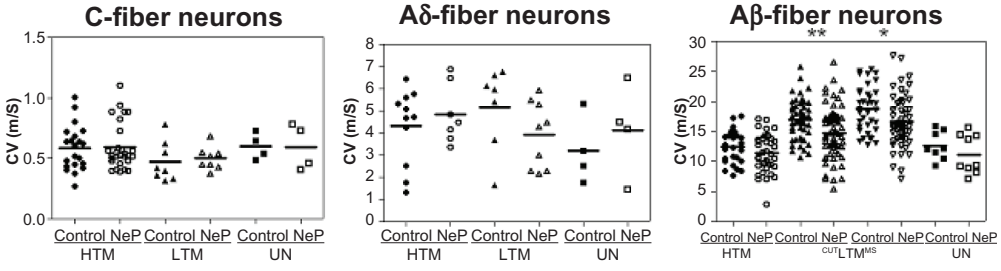

Figure 5 Comparison of dorsal root conduction velocity of dorsal root ganglion neurons between control and neuropathic rats. Scatter plots show the distribution of variables with the median (horizontal line) superimposed in each case. Details are the same as in Figure 2.

Notes: Asterisks above the graph indicate the significant difference between control and neuropathic animals: *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$. The absence of an asterisk indicates lack of a statistically significant difference.

In Aδ-fiber neurons, conduction velocity was 4.32 ± 0.514 m/sec (n = 11) and 4.86 ± 0.505 m/sec (n = 7) in control and neuropathic high threshold mechanoreceptor neurons, respectively ($P = 0.4871$), while it was 5.17 ± 0.705 m/sec (n = 7) and 3.91 ± 0.500 m/sec (n = 9) in control and neuropathic low threshold mechanoreceptor neurons, respectively ($P = 0.1548$) and was 3.21 ± 0.766 m/sec (n = 3) and 4.14 ± 1.041 m/sec (n = 3) in control and neuropathic unresponsive neurons, respectively ($P = 0.4966$).

In Aβ-fiber neurons, conduction velocity was 12.42 ± 0.548 m/sec (n = 26) in control high threshold mechanoreceptor neurons and 11.38 ± 0.504 m/sec (n = 39) in neuropathic high threshold mechanoreceptor neurons ($P = 0.1794$), 17.02 ± 0.469 m/sec (n = 50) and 14.70 ± 0.548 m/sec (n = 63) in control and neuropathic cutaneous low threshold mechanoreceptor neurons, respectively ($P = 0.0023$), 18.76 ± 0.638 m/sec (n = 36) and 16.69 ± 0.599 m/sec (n = 53) in control and neuropathic low threshold mechanoreceptor muscle spindle neurons, respectively ($P = 0.0235$), and 12.67 ± 0.831 m/sec (n = 8) in control versus 11.15 ± 1.088 m/sec (n = 9) in neuropathic unresponsive neurons ($P = 0.2922$).

Activation of sensory receptors

The activation threshold of sensory receptors associated with different neuron types was studied throughout the entire hind leg. This was done to complement the data reported above regarding properties of the soma and the axon. Almost all of the neurons studied with identifiable receptive fields could be classified into one of the categories according to the type of stimulus; Table 2 shows the locations of the receptive fields of the neurons recorded.

C-fiber neurons in neuropathic rats did not show differences in the threshold of activation of the receptive field compared with those in control rats. C-fiber low threshold mechanoreceptor neurons (n = 8 in control; n = 8 in neuropathic rats) were activated by slow brushing on the receptive field or lightly stretching of the skin surrounding the receptive field. Neither stimulus produced any discharge in C-fiber high threshold mechanoreceptor neurons (n = 21 in control rats, n = 28 in neuropathic rats), which was activated only by stimuli in the noxious range. The distributions of C-fiber high threshold mechanoreceptor and low threshold mechanoreceptor neuron receptive fields were relatively evenly distributed over the hind leg, each with relatively small receptive field sizes.

Similarly, none of the Aδ-fiber neurons in neuropathic rats showed any difference in activation of the receptive field when data from control and neuropathic rats were compared. For Aδ-fiber high threshold mechanoreceptor neurons (n = 11 in control, n = 7 in neuropathic rats), the receptive fields usually consisted of several small spots and were found on the foot, calf, and thigh. All responded to noxious stimuli.

Aδ-fiber low threshold mechanoreceptor neurons tested in this way were D-hair neurons (n = 7 in control; n = 9 in neuropathic rats). They were activated by von Frey filaments (≤0.16 g) and were found on the foot, calf, and thigh.

Table 2 Locations of receptive fields of high-threshold mechanoreceptors and low-threshold mechanoreceptors in controls and neuropathic rats

CV range	Receptor class	Model	n	Foot	Calf	Thigh	Ankle joint	Knee joint
C	HTM	Control	21	8	5	4	2	2
		NeP	28	12	4	6	4	2
	LTM	Control	8	5	2	1	/	/
		NeP	8	6	/	2	/	/
Aσ	HTM	Control	11	5	3	3	/	1
		NeP	7	4	2	1	/	/
	LTM	Control	7	2	3	2	/	/
		NeP	9	2	4	3	/	/
Aβ	HTM	Control	26	4	6	8	4	4
		NeP	39	3	9	12	3	2
	LTM (GF)	Control	20	4	6	8	1	1
		NeP	25	3	5	13	2	1
	LTM (RA)	Control	20	10	1	2	1	2
		NeP	24	9	1	3	2	1
	LTM (SA)	Control	10	9	/	/	1	/
		NeP	14	12	/	/	2	/
	LTM (MS)	Control	36	2	25	9	/	/
		NeP	53	3	40	10	/	/

Abbreviations: HTM, high threshold mechanoreceptive neurons; LTM, low threshold mechanoreceptive neurons; MS, muscle spindle neurons; RA, rapidly adapting neurons; SA, slowly adapting neurons; GF, guard/field neurons.

For Aβ-fiber high threshold mechanoreceptor neurons (n = 26 in control rats, n = 39 in neuropathic rats), the distribution of receptive fields was also over the entire hind leg. All responded to the noxious stimuli, including noxious stimulation deeply toward the bone. Aβ-fiber low threshold mechanoreceptor neurons and guard/field hair neurons (n = 20 in control rats, n = 25 in neuropathic rats) were distributed entirely over hairy skin and were activated by lightly stimulating the tip of the hair/moving a group of hairs. Rapidly adapting glabrous neurons (n = 6 in control rats, n = 6 in neuropathic rats) were activated by lightly stimulating the glabrous skin of the rat foot with blunt objects; these neurons responded to these stimuli with a brief, quickly adapting discharge. Pacinian neurons (n = 14 in control rats, n = 18 in neuropathic rats) were activated by gently tapping on the experiment table and were found over the entire hind leg. Slowly adapting neurons (n = 10 in control rats, n = 14 in neuropathic rats) were activated by lightly stimulating narrow skin strips surrounding the nails in the foot, except for one control neuron with a receptive field on the ankle joint. muscle spindle neurons were activated by touching along the muscle belly or changing joint position. One particular abnormality observed was found in two Aβ-fiber neurons in neuropathic rats; in response to current injection into the cell body, the response of these neurons (n = 2) resembled that of muscle spindle neurons or slowly adapting neurons in terms of relatively slow adaptation, but the receptive-field characteristics of these neurons were similar to other hair neurons with low thresholds to stimulation of hairs (≤0.008 g von Frey filament stimulation; these neurons are not included in this study).

Previous studies have shown that the threshold of activation of most nociceptors in control rats is higher than 14 mN (1.43 g).[30] To identify whether high threshold mechanoreceptor neurons showed any differences in mechanical sensitivity in this model, we also measured the mechanical threshold of a selected number of high threshold mechanoreceptor neurons in control and neuropathic rats. The mechanical threshold for C-fiber high threshold mechanoreceptor neurons was within the range of 2–100 g in control rats (n = 14) versus 6–100 g in neuropathic rats (n = 15). Mechanical thresholds for Aδ-fiber high threshold mechanoreceptor neurons were in the range 4–60 g in control rats (n = 8) versus 4–60 g in neuropathic rats (n = 6). Mechanical thresholds for Aβ-fiber high threshold mechanoreceptor neurons were in the range of 4–60 g in control rats (n = 8) versus 2–100 g in neuropathic rats (n = 6). None of the high threshold mechanoreceptor neurons in neuropathic rats showed a mechanical threshold of activation below 1.4 g.

Discussion

Classification of dorsal root ganglion neurons

The aim of the present study was to compare primary sensory neurons in control and neuropathic rats in order to identify changes in peripheral neurons associated with tactile hypersensitivity. Our interest was particularly in the type of neuron exhibiting change. To achieve this aim, dorsal root ganglion sensory neurons were classified during intracellular in vivo electrophysiological experiments according to parameters reported previously by other laboratories to distinguish dorsal root ganglion neuron types, including the configuration of the action potential, conduction velocity, and response properties to application of natural stimuli to peripheral receptive fields, such as threshold of activation and adaptation.[25–30] With this classification, each dorsal root ganglion neuron could be functionally classified and each could be distinguished as nociceptive (high threshold mechanoreceptor), non-nociceptive (low threshold mechanoreceptor), or unresponsive. The conduction velocity criteria for classification in the present study were based on those described previously by Lawson et al.[27–29] These criteria were followed because the experimental approach in the present study most closely approximated those in Lawson's previous reports, including similar surgical procedures, recording techniques, and recording setups.

Although differentiation of Aβ-fiber, Aδ-fiber, and C-fiber neurons was based on the conduction velocity ranges in this study, as reported by others,[25–29] there is a potential overlap between these three main neuron subtypes if the conduction velocity changes as a result of nerve injury. The concern here is that an increase in excitability could lead to an increase in conduction velocity, so that some Aδ-fiber and C-fiber neurons would conduct faster and thus be classified as Aβ neurons. However, our current study and a previous study from another group[33] showed that conduction velocity was slower in the neuropathic model studied. As a result, faster conducting neurons might exhibit slower conduction velocities and thus be classified as Aδ-fiber or C-fiber neurons. It should be noted, then, that Aδ-fiber and C-fiber neurons would not be classified as Aβ-fiber neurons. Furthermore, our data showed that Aβ-fiber, Aδ-fiber, and C-fiber neurons in the cuff model of neuropathic pain still conducted in three clearly separate ranges.

The differentiation between the high threshold mechanoreceptor neurons and low threshold mechanoreceptor neurons in control rats was also clearly maintained in this neuropathic animal model. In neuropathic rats, high threshold mechanoreceptor neurons in the C-fiber, Aδ-fiber, and Aβ-fiber neuron ranges exhibited a relatively depolarized resting membrane potential, a relatively higher action potential amplitude, relatively slower action potential kinetics (ie, longer action potential duration, action potential rise and fall times, and slower action potential maximum rising and falling rates), a relatively higher AHP amplitude, and a longer 50% AHP recovery time than that exhibited by low threshold mechanoreceptor neurons. Thus, other than the few neurons that are described in the results and excluded in this classification, all neurons were clearly classified in both control and neuropathic rats.

Differences in properties of neuron types

In the present study, when comparing control and neuropathic animals, none of the small diameter C-fiber dorsal root ganglion neurons with identifiable receptive fields exhibited any differences in any of the properties investigated here, including both low threshold mechanoreceptor and high threshold mechanoreceptor neurons. In contrast, in the present study, dorsal root ganglion A-type neurons, in particular Aβ-fiber low threshold mechanoreceptor neurons in neuropathic rats, showed significant differences in electrophysiological properties compared with those in control animals, manifesting as decreased conduction velocity, a more depolarized Vm, and slower action potential kinetics.

Our results, demonstrating changes mainly in large diameter primary afferent neurons, are compatible with those of several groups investigating other types of rat model, which showed that most ectopic discharge is generated in large-diameter, fast-conducting myelinated Aβ-fiber neurons after nerve injury.[9,34–37] In fact, some features such as the longer action potential duration, rise time and fall time, and lack of AHP changes in A-fiber neurons in our neuropathic rats are similar to the well described differences in A-fiber neurons after peripheral axotomy[36,38–41] and neighboring intact dorsal root ganglion neurons recorded in L5 neurons in the spinal nerve ligation model of peripheral neuropathy.[36] The decreased conduction velocity of A-fiber neurons is similar to that reported in A-fiber neurons after chronic constriction injury of the sciatic nerve.[33] Somewhat different magnitudes of change in these parameters in the different reports might be attributable to many factors, such as different sensory neurons targeted ("injured" versus "intact" neurons) and to different animal models employed (eg, nerve-section axotomy model, spinal nerve ligation model, peripheral nerve section model, chronic constriction injury model, and compression of the dorsal root ganglion model). Different recording techniques, such as sharp microelectrodes in vivo or in vitro in some experiments versus patch electrodes to study dissociated cells in others, and different animal species chosen, strain, age, gender, and time between the initial model induction and electrophysiological experiment may have also contributed to differences in the magnitude of the changes in different reports.[38]

Conduction velocities and action potential properties of unresponsive neurons were not significantly different from those of high threshold mechanoreceptor units in the C-fiber, Aδ-fiber, and Aβ-fiber ranges, both in control and neuropathic animals. These types of neuron were thus probably high threshold mechanoreceptor neurons with inaccessible receptive fields or were very high threshold mechanoreceptor units, as described earlier by Lawson et al.[29] However, although unresponsive neurons in neuropathic animals undergo electrophysiological changes compared with control animals, measurement of mechanical sensitivity was not possible because these neurons were not responsive to any mechanical stimulation of the cutaneous receptive field, and thus they might not be related to tactile allodynia.

Novel contribution to the literature

One point of differentiation between the present study and previous literature is that we used the Mosconi and Kruger model, whereas previous reports were based on the chronic constrictive injury model, Chung model, peripheral nerve cut model, and dorsal root ganglion compression model. In the Mosconi and Kruger model, there is no peripheral axotomy, no dorsal root axotomy, no physical damage to the dorsal root ganglion through compression, and all neurons are classified on the basis of their response to activation of their respective receptive fields using natural stimuli. The Mosconi and Kruger model most closely resembles the chronic constrictive injury model in these respects.

As pointed out earlier, many previous studies were based on axotomized neurons that were anatomically and/or only partially functionally identified. Identifying axotomized dorsal root ganglion neurons as nociceptive or non-nociceptive[39,42,43] on the basis of conduction velocity alone is less valid than activating neurons from their respective receptive fields because some slowly conducting sensory neurons are non-nociceptive and some fast conducting sensory neurons are nociceptive.[29] Koerber et al[44] cut

peripheral sensory nerves and allowed time for regeneration to peripheral tissues; however, although they activated sensory neurons by peripheral stimulation, it is unclear whether the neurons retained their original nociceptive or non-nociceptive properties after the regeneration process, and the focus of the study was on plasticity of central projections of sensory neurons after axotomy, and there was no attempt to focus specifically on the possible participation of Aβ-fiber neurons in nociception following peripheral neuropathy.

Another point of differentiation between the present study and several of the earlier studies is that our data were derived from in vivo recordings made from dorsal root ganglion sensory neurons. Some previous studies[45–48] were based on in vitro recording raising a similar concern about accurately classifying neurons as nociceptive versus non-nociceptive. Yet other studies were run in vivo in the anesthetized rat model of peripheral neuropathy based their classification of neurons on the basis of conduction velocity alone.[9,34] Xie et al[49] recorded from dorsal root ganglion neurons in vivo and reported functional changes in the chronic constrictive injury model. However, while they studied responses of neurons to thermal stimuli, there was no classification based on responses to low or high threshold mechanical stimulation, and neurons were not classified as nociceptive versus non-nociceptive. Zhao et al[50] recorded from sensory axons in the chronic constrictive injury model in vivo and reported spontaneous ectopic discharge in these neurons. However, the neurons were not functionally classified and therefore no information was provided on the neuron type that displayed such changes.

Therefore, in the present study, a number of methodological approaches were used that differed from those reported previously. Further, a novel contribution is that this is the first to report changes in fully classified sensory neurons on the basis of their responses to natural stimuli to the receptive fields in vivo in an animal model in which axons were not cut.

Correlation of changes in Aβ-type low threshold mechanoreceptors with tactile hypersensitivity

While this evidence argues against C-fiber neurons contributing to tactile allodynia after induction of the model, this has sometimes been assumed to involve high threshold mechanoreceptor units as a possible contribution. Similarly, while there is evidence for Aβ-fiber neurons contributing to tactile allodynia after nerve or tissue injury, this has sometimes been assumed to involve low threshold mechanoreceptor units as a possible contribution.[5]

Action potential configuration, conduction velocity, and peripheral activation threshold of all subtypes of dorsal root ganglion neuron were systematically evaluated in this study, because each of these parameters might reflect changed electrophysiological properties in various parts of the primary sensory neuron, such as the soma, the axon, and the receptive field, respectively. These features of peripheral neurons have been previously described and have been thought to play a role in generating the pain signal in several animal models of peripheral neuropathy.[5,7,14,15]

Although it is widely believed that afferent C-fiber input is a necessary condition for the induction and maintenance of central sensitization, none of the C-fiber neurons in this study showed any difference in electrophysiological properties. Perhaps most important, no C-fiber high threshold mechanoreceptor neurons showed any decrease in mechanical sensitivity when tested with von Frey filaments.

On the other hand, A-type neurons, especially Aβ-fiber low threshold mechanoreceptor neurons in neuropathic rats, showed significant differences in electrophysiological properties. One of the changes was a decrease in conduction velocity, possibly due to demyelination. It has been suggested that in models of peripheral neuropathic pain, demyelination leads to crosstalk whereby non-nociceptive afferents can activate nociceptive afferents.[51]

Our previous studies in this same model showed nociceptive responses to innocuous mechanical stimulation in a wide dynamic range of spinal neurons,[19] and it was further proposed that ectopic activity recorded from these neurons was mediated via myelinated afferents.[20] Thus, the present results provide further support for a possible role of Aβ-fiber low threshold mechanoreceptors in the behavioral tactile hypersensitivity exhibited in this model, because these neurons remained connected to their normal impulse generating site and respond to normal innocuous mechanical stimuli, which are essential for defining tactile sensitivity.

Our finding adds to the body of evidence that C-fiber dorsal root ganglion neurons are likely not related to tactile allodynia,[52,53] and raise the possibility that low threshold mechanoreceptor neurons of Aβ-fiber dorsal root ganglion neurons fulfill this role. The mechanisms underlying the electrophysiological changes in Aβ-type low threshold mechanoreceptor neurons that could induce neuropathic pain or allodynia remain to be determined. One possible explanation is that some Aβ-type low threshold mechanoreceptor neurons undergo phenotypic changes and take up a new role in nociception,

and began to convey signals along novel pathways leading to activation of spinal nociceptive mechanisms.[5] There is evidence that mediators released, such as substance P, calcitonin gene-related peptide, and brain-derived neurotrophic factor, as well as neuropeptide Y released by A-type neurons, might trigger changes in the responsiveness of postsynaptic neurons and rewiring of sensory pathway at the first sensory synapse in the spinal cord.[54–60]

We speculate that there may be an altered supply of such factors in the neurons that have undergone the changes reported here. These changes may therefore constitute a mechanism leading to the pain, dysesthesia, and allodynia that commonly accompany peripheral neuropathy.

Cellular mechanisms of underlying differences in A-type neurons

These differences in neurons in neuropathic animals might be due to membrane remodeling, thus altering the intrinsic electrogenic properties of the neuronal membrane in those neuron types exhibiting changes. There are three major ion channels, ie, Na^+, Ca^{2+} and K^+, which play major roles in determining electrogenic properties of neurons. For example, Na^+ channels can modulate the resting membrane potential in dorsal root ganglion neurons, and Na^+ channels are also likely to have a major influence on the action potential rising phase and therefore on the duration of the action potential.[61] Ca^+ inward currents likely contribute to the falling phase inflections in high threshold mechanoreceptor neurons.[27,62] K^+ channels have been reported to contribute to the repolarization phase in low threshold mechanoreceptor neurons.[27] Differences in expression and/or activation of both voltage-gated and Ca^{2+}-activated K^+ channels have been reported as likely to contribute to the AHP.[27] Thus, alterations in the level of expression, cellular localization, and distribution or activation/kinetics of each of these ion channel types might lead to the changes in action potential configuration in neuropathic rats.

Changes in the activity of sodium channels and the consequences of these changes have been reported in various neuropathic pain models. Immunohistochemical studies have demonstrated a reorganization of the levels of expression and distribution of various sodium channels in neuropathic animal models.[14,63,64] The expression of some sodium channel subtypes in dorsal root ganglion cell bodies is diminished following nerve injury, while others appear de novo and yet others are distributed to different parts of the neuron.[65] Changes in the activity of calcium channels and potassium channels have also been reported.[66] For example, voltage-clamp studies of isolated currents in dissociated axotomized dorsal root ganglion cells have revealed an upregulation of a tetrodotoxin-sensitive Na^+ current and a downregulation of a tetrodotoxin-resistant Na^+ current,[61,67–69] together with a reduction of the K^+ and Ca^{2+} currents.[70–72]

Explanations for the mechanisms underlying these changes in membrane channel expression might be based on many factors, such as nerve growth factor or glial cell line-derived neurotrophic factor, cytokines, or other inflammatory mediators released by immune cells and Schwann cells.[73–79] Nerve growth factor and glial cell line-derived neurotrophic factor are important neurotrophic factors for maintaining normal function of sensory neurons and may influence action potential electrogenesis and neuronal excitability in dorsal root ganglion neurons via regulating ionic currents.

Demyelination, which has been proposed to result in membrane remodeling, should also be considered as a possible driving force of change, because demyelination has been reported to lead to ion channel redistribution.[80] In normal conditions, newly synthesized Na^+ channels are transported in endocytoplasmic vesicles along the axons to be expressed only at specific target sites, such as nodes of Ranvier and nerve terminal endings. As a consequence of the neuronal damage in this model, this target-specific transfer may be altered and the channels in transit may be redistributed in any remaining part of the membrane, particularly in dorsal root ganglions and demyelinated patches.[66] In fact, our data show that the decreased conduction velocity of Aβ low threshold mechanoreceptor neurons might be due to demyelination so as to be compatible with the change in action potential shape in these neurons following peripheral neuropathy.

Some of these findings or other factors might be contributing in various ways to the changes we have observed in the shape of the action potential of different A-type neurons. In fact, the more heavily myelinated Aβ-fiber low threshold mechanoreceptors in this neuropathic model might be more affected by such comprehensive factors and thus showed significant changes in functional properties.

Conclusion

The purpose of this study was to examine the electrophysiological properties and mechanical sensitivity of the different types of dorsal root ganglion neurons in control versus neuropathic animals. Comparing previous reports, this is the first study providing evidence showing changes in functional properties of sensory dorsal root ganglion neurons with identifiable receptive fields. In this model,

there were no differences in C-fiber neurons between control and model animals. However, there were significant and possibly important differences in dorsal root ganglion neurons associated with Aβ-fibers and Aδ-fibers, especially Aβ low threshold mechanoreceptor neurons. These findings are unique and unexpected because Aβ low threshold mechanoreceptor neurons are normally considered to be non-nociceptive neurons. We interpret these data to suggest that A-type but not C-type primary sensory neurons in this model of peripheral neuropathy may be involved in generating the tactile hypersensitivity seen in these animals in the von Frey behavioral test.

Disclosure

The authors report no conflicts of interest in this work.

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