Electrophysiological differences between nociceptive and non-nociceptive dorsal root ganglion neurones in the rat *in vivo*

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Intracellular recordings were made from 1022 somatic lumbar dorsal root ganglion (DRG) neurones in anaesthetized adult rats, classified from dorsal root conduction velocities (CVs) as C, Aδ or Aα/β, and according to their responses to mechanical and thermal stimuli as nociceptive (including high-threshold mechanoreceptive (HTM) units), and non-nociceptive (including low-threshold mechanoreceptive (LTM) and cooling units). Of these, 463 met electrophysiological criteria for analysis of action potentials (APs) evoked by dorsal root stimulation. These included 47 C-, 71 Aδ- and 102 Aα/β-nociceptive, 10 C-, 8 Aδ— and 178 Aα/β-LTM, 18 C- and 19 Aδ- unresponsive, and 4 C-cooling units. Medians of AP and afterhyperpolarization (AHP) durations and AP overshoots were significantly greater for nociceptive than LTM units in all CV groups. AP overshoots and AHP durations were similar in nociceptors of all CV groups whereas AP durations were greater in slowly conducting, especially C-fibre, nociceptors. C-cooling units had faster CVs, smaller AP overshoots and shorter AP durations than C-HTM units. A subgroup of Aα/β-HTM, moderate pressure units, had faster CVs and AP kinetics than other Aα/β-HTM units. Of the Aα/β-LTM units, muscle spindle afferents had the fastest CV and AP kinetics, while rapidly adapting cutaneous units had the slowest AP kinetics. AP variables in unresponsive and nociceptive units were similar in both C- and Aδ-fibre CV groups. The ability of fibres to follow rapid stimulus trains (fibre maximum following frequency) was correlated with CV but not sensory modality. These findings indicate both the usefulness and limitations of using electrophysiological criteria for identifying neurones acutely *in vitro* as nociceptive.

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Dorsal root ganglion (DRG) neurones convey somatosensory information as action potentials (APs) to the CNS. These neurones are of two main types: non-nociceptive neurones that respond to non-noxious, low intensity, normally non-painful stimuli; and nociceptive neurones that respond to noxious, high intensity, normally painful stimuli. DRG neurones are heterogeneous in their conduction velocities (CVs), receptive properties (for review see Lawson, 2002) and somatic AP configuration (Yoshida *et al.* 1978; Gorke & Pierau, 1980; Harper & Lawson, 1985; Djouhri *et al.* 1998).

Following the first report of a distinct functional relationship between AP configuration and afferent receptor properties of cat nodose ganglion neurones (Belmonte & Gallego, 1983), such relationships have been described in snake trigeminal ganglia (Terashima Si & Liang 1994) and DRG neurones of several species. Nociceptive DRG neuronal somata tend to have broader APs than low-threshold mechanoreceptive (LTM) units in the same CV group. This was demonstrated for A-fibre neurones in cat (Koerber *et al.* 1988), rat (Ritter & Mendell, 1992) and guinea-pig (Djouhri *et al.* 1998), for C-fibre neurones in guinea-pig (Djouhri *et al.* 1998), and for afferent C-fibres in pig and rat (Gee *et al.* 1999). In contrast, in cat DRGs, both C-nociceptive and C-LTM neurones were reported to have broad somatic APs (Traub & Mendell, 1988).

Thus the only study in rat DRGs of the relationship between somatic AP variables, including after-hyperpolarization (AHP) configuration, and sensory properties *in vivo* was limited to A-fibre nociceptive and LTM neurones (Ritter & Mendell, 1992) with no such study either on C-fibre neurones or on functional subtypes of nociceptive or LTM neurones within any CV group. This is because for the latter study very large

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numbers of neurones are required. The importance of establishing the relationship between somatic AP configuration and sensory modality for C-fibre neurones in rat is the possible species variability in C-fibre AP duration (see above), and the reliance of many studies of dissociated rat DRG neurones *in vitro* on AP shape (and cell size) for identification of putative nociceptors.

Finally, an important property of sensory neurones is the ability of their fibres to carry trains of APs, the rate of which (fibre-following frequency, FFF) influences the CNS and is dictated by the membrane properties of that neurone. It is not known how FFF is related to sensory receptor properties, AP configuration and CV in nociceptive and non-nociceptive neurones.

We have therefore used intracellular recordings from the somata of DRG neurones *in vivo* to examine whether there are: (1) correlations between somatic AP configuration and receptor modality in C-fibre neurones in the rat; (2) differences in somatic spike shape in different types of non-nociceptive and nociceptive neurones of all CVs; and (3) correlations between FFF and sensory receptor type. Such knowledge of the normal electrophysiological properties of DRG neurones is important as a basis for understanding any changes in their properties in chronic pain states.

Methods

Animal preparation

Experiments were carried out on young female Wistar rats (weight, 150–180 g). Experimental procedures conformed to the UK Animals (Scientific Procedures) Act 1986. Rats were anaesthetized with an initial dose of 70–80 mg kg^{-1} I.P. sodium pentobarbitone that produced deep anaesthesia with areflexia (i.e. total absence of limb withdrawal reflex). A tracheotomy was performed to allow artificial ventilation and end-tidal CO_2 monitoring. End-tidal CO_2 was kept around 3–4% by adjusting the rate and stroke volume of the respiratory pump. The left carotid artery and external jugular vein or the right carotid artery were cannulated to, respectively, enable regular injections of the additional doses of the anaesthetic (10 mg kg^{-1}) that were required to maintain this deep level of anaesthesia and to allow monitoring of blood pressure. The temperature in the paraffin pool measured near the DRG under study was maintained throughout at 30 ± 2°C; because we have found that with the large heat loss from the extensive pool, it is hard to stabilize the pool temperature at 37°C without the core temperature oscillating to above 37°C, causing reduction in viability of the preparation. The left hindlimb was extended and fixed with superglue to the underneath (hairy) foot surface for stability during sensory testing, leaving the glabrous foot surface facing upwards, and causing poor access to the dorsal surface of the foot. Full details of the animal preparation were as reported previously in guinea-pig (Lawson *et al.* 1997; Djouhri *et al.* 1998) and rat (Fang *et al.* 2002).

All animals were paralysed with a muscle relaxant, pancuronium (0.5 mg kg^{-1}, I.A.) to improve stability during recording. The muscle relaxant was always accompanied by an additional dose (10 mg kg^{-1}, I.A.) of the anaesthetic and the two substances were administered at regular intervals (approximately hourly). The blood pressure remained stable at above 80 mmHg throughout showing no indication of any reduction in the depth of anaesthesia at any stage in all the present experiments. These anaesthetic doses were the same as those that induced deep areflexic anaesthesia during the period (2–3 h) of animal preparation.

Intracellular recordings

Intracellular recordings from the neuronal somata in the left L3–L6 DRG (mainly L4 and L5) were obtained using glass microelectrodes filled with either KCl (3M) or a fluorescent dye. The dyes used were Lucifer yellow (LY) in 0.1 M LiCl, ethidium bromide (EB) in 1 M KCl or occasionally Cascade Blue (CB) as a 3% solution in 0.1 M LiCl. Cells were impaled by advancing the microelectrode in 1-μm steps and applying a small capacitance buzz until a membrane potential (E_m) was seen. Somatic APs were antidromically evoked by dorsal root stimulation (through bipolar platinum electrodes) with single rectangular pulses (0.03 ms duration for A-fibre units or 0.3 ms for C-fibre units). The stimulus intensity (up to 25 V) was adjusted to twice threshold for A-fibre units and between one and two times threshold for C-fibre units. Somatic APs were recorded on-line with a CED (Cambridge Electronics Design) 1401 plus interface and the SIGAV, SIGNAL or Spike II programs from CED and were subsequently analysed off-line using CED Spike II program.

Action potential variables

AP variables including AP duration at base (AP duration), AP rise time (RT), AP fall time (FT), AP overshoot and the duration of the afterhyperpolarization (AHP) to 80% recovery were measured for each unit as previously described (see Djouhri *et al.* 1998; Fig. 1). As previously described (Djouhri & Lawson, 2001*b*), the term AP overshoot refers to the position of the AP apex relative to 0 mV. Thus an AP that does not reach 0 mV is a non-overshooting AP and is shown as a negative value on graphs (see Fig. 3). CV, E_m, AP height, maximum rate of rise (MRR), maximum rate of fall (MRF) and AHP amplitude were also measured (see Tables 1–3). At the end of each experiment, animals were killed with an overdose of anaesthetic and the conduction distance was measured from the cathode of the stimulating electrode pair to the approximate (± 0.25 mm) location of the neurone in the DRG as previously described (Djouhri & Lawson, 2001*a*). The conduction distance (4.5–13 mm)

and latency to onset of the evoked somatic AP were used to estimate the CV of each unit. Utilization time was not taken into account. Dorsal root CVs were classed as C (≤ 0.8 m s^{-1}), Aδ (1.5–6.5 m s^{-1}) and Aα/β (> 6.5 m s^{-1}). These borderlines were based on recordings of C, Aδ and Aα/β waves in compound APs from dorsal roots that were made under the same conditions as those of intracellular recordings (i.e. at the same temperature range in the paraffin pool), and from rats of the same sex and weight range (Fang *et al.* 2002). Differences between these values and those in some other studies in rodents are discussed later (see Discussion).

Fibre-following frequency

In order to determine whether maximum fibre-following frequency (FFF) was related to the sensory receptor type, the FFF of dorsal root fibres were tested in some neurones as previously described in detail (Djouhri *et al.* 2001). Briefly, the dorsal roots were stimulated with trains of electrical stimuli at frequencies of 10–800 Hz and evoked somatic potentials were recorded intracellularly. The duration of all the trains was 200 ms, and the frequency of stimulation was gradually increased from 0.33 Hz with a short pause of at least 4 s between trains. Increasing the stimulus frequency resulted in conduction failure of some of the APs, judged by the presence of non-invading APs (electrotonically conducted responses) and/or by the complete absence of evoked potentials in the soma. These electrotonic responses/potentials are thought to reflect propagating APs in the fibre that fail to invade the soma presumably at the sites of low safety factor such as the T-junction or at the junction of fibre and soma (Luscher *et al.* 1994). It seems likely that failure can occur at both these sites, as sometimes three different-sized spikes (a full height spike and two different-sized partial height spikes) are seen in the same neurone (Djouhri *et al.* 2001). The FFF was the maximum frequency at which each stimulus resulted in a detectable evoked response of any size in the soma. For each neurone, the percentage of the stimuli that evoked a response in the soma was plotted against stimulation frequency for a range of frequencies. From this, the frequency at which 80% of the stimuli were followed by evoked somatic responses was calculated (FFF).

Sensory receptive properties

As previously described in the guinea-pig (Lawson *et al.* 1997; Djouhri *et al.* 1998) and rat (Fang *et al.* 2002) the sensory receptive properties of DRG neurones were identified as follows. The receptive fields (RFs) of all units were located with non-noxious (innocuous) mechanical and/or with intense (noxious) stimuli that are potentially damaging to the tissue. The cooling stimuli used were very brief; a spray of ethyl chloride, a cooled metal rod or ice. Low-threshold cooling units showed ongoing activity at room temperature that was inhibited by radiant warming and they then responded more vigorously to these cooling stimuli, but not to mechanical stimuli. High-threshold mechano-cold (MC) units also responded to these cooling stimuli as well as noxious mechanical stimuli but did not show ongoing firing at room temperature. Skin temperature was not measured. The non-noxious mechanical stimuli included light brushing of limb fur, skin contact and light pressure with blunt objects, light tap, tuning forks vibrating at 100 or 250 Hz and pressure with calibrated von Frey hairs. If no response to these mechanical stimuli was seen, noxious mechanical stimuli were applied with a needle, fine forceps or coarse toothed forceps and a noxious heat was applied with hot water at 50–65°C from a 5 ml syringe (with no needle). In general, these noxious stimuli would be classed as painful when applied to human skin.

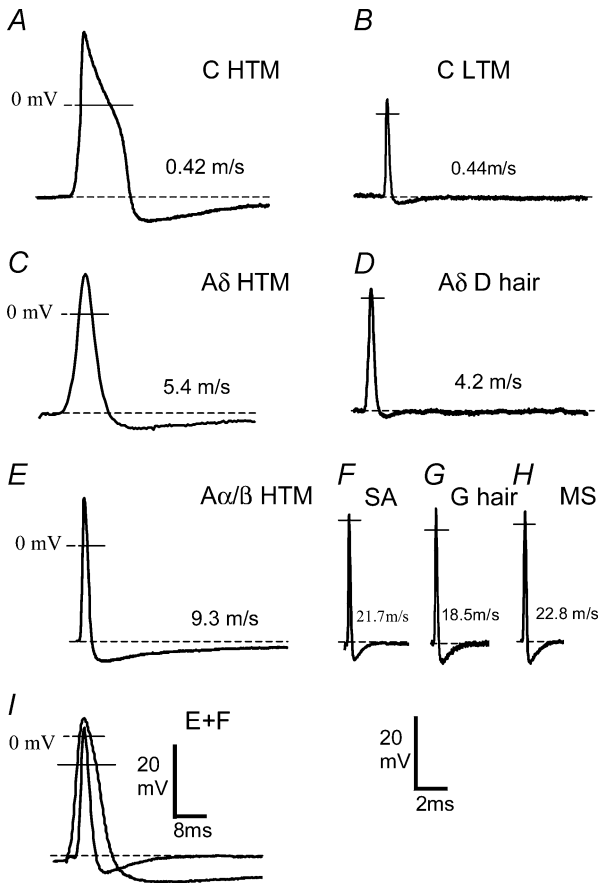

Figure 1. Typical somatic APs of nociceptive and LTM neurones with C, Aδ- or Aα/β-fibres
The APs were evoked antidromically by dorsal-root stimulation and recorded intracellularly from C- (*A* and *B*), Aδ- (*C* and *D*) and Aα/β-fibre (*E–H*) DRG neurones with different sensory receptive properties. The CVs and types of neurone are shown. The horizontal solid lines represent 0 mV. *I*, expanded time base and superimposed traces of *E* and *F* to show that as in the C- and Aδ-fibre CV ranges, nociceptors with Aα/β fibres have longer AP and AHP durations and greater AP overshoots than LTM units. APs were chosen because they had values close to the medians for the main variables (AP, AHP duration and AP overshoot).

Table 1. Comparison of electrophysiological properties of nociceptive and non-nociceptive DRG neurones

CV range	Receptor class	Total sample (*n*)	% of neurones	CV of total sample (m s^{-1})	Electro sample (*n*)	E_m (–mV)	AP height (mV)	MRR (mV ms^{-1})	MRF (mV ms^{-1})	AHP amplitude (mV)
C										
	NOCI	82	54	0.44 (0.36–0.54)	47 (43)	50 (59–42)	75 (64–85)	222 (142–319)	93 (68–138)	8.3 (4.8–10.8)***
	HTM	66	43	0.42 (0.35–0.54)	40 (37)	51 (58–42)	75 (65–85)	222 (137–319)	92 (67–138)	8.3 (5.5–11.1)
	MH	9	6	0.39 (0.38–0.61)	5 (5)	52	75	185	112	3.7
	MC	7	5	0.48 (0.39–0.65)	2 (1)	61	71	274	109	10.7
	NON-NOCI	17	12		14					
	LTM	13	9	0.44 (0.34–0.55)	10 (10)	57 (67–47)	61 (52–79)	145 (114–597)	89 (61–121)	3.8 (2.8–5.1)
	COOL	4	3	0.57 (0.49–0.67)*	4 (2)	54	52	193	72	6.7
	UNRES	52	34	0.44 (0.39–0.58)	18 (15)	57(63–41)	77 (60–95)	182 (107–257)	79 (57–120)	6.8 (3.9–8.6)
	Total	151	100		79 (70)					
C/Aδ										
	NOCI	3	50	1.03	3	63	64	184	77	6.5
	UNRES	3	50	1.04	3	50	93	208	99	5.1
	Total	6	100		6					
Aδ										
	NOCI	77	58	4.7 (3.5–5.6)	71	51 (57–46)	71 (61–87)	245 (182–347)	147 (97–214)	8.9 (6.8–10.9)
	HTM	71	53	4.9 (3.5–5.7)	67	52 (57–46)	71 (61–88)	248 (183–357)	148 (97–216)	8.9 (6.9–11.20)
	MH	2	2	3.6	2	59	65	192	98	7.5
	MC	4	3	3.6 (3.5–4.5)	2	47	58	177	89	7.2
	NON-NOCI									
	LTM	25	19	3.6 (2.8–4.9)	8	57 (65–51)	65 (57–68)	256 (207–329)	157 (121–208)	7.9 (5.2–10.3)
	UNRES	31	23	4.1 (3.1–5.2)	19	55 (59–50)	68 (61–76)	261 (195–315)	172 (140–253)	6.9 (5.1–8.4)
	Total	133	100		98					
Aα/β										
	NOCI	237	32	11.8 (8.7–15.7)***	102	58 (65–51)	77 (67–90)***	331 (247–503)	195 (137–275)	9.2 (7.1–11.5)***
	MP (HTM)	26	3.0	14.8 (11.3–16.9)*	13	56 (65–50)	76 (59–87)	348 (258–570)	198 (155–292)	10 (8.2–13.3)
	Other HTM	204	28	11.5 (8.5–15.5)	86	58 (64–51)	76 (67–92)	340 (245–503)	197 (133–276)	9.1 (6.8–11.2)
	MH	4	0.5	14.0 (10.5–14.6)						
	MC	3	0.5	18.9	3	63 (69–58)	89	318	144	8.1
	NON-NOCI									
	LTM	495	67	16.6 (12.7–21.3)	178	59 (66–54)	72 (63–79)	426 (301–713)	235 (169–382)	7.8 (4.9–10.5)
	Total	732	100		280					

The numbers of neurones in the total sample (*n*) given in the third column were used for analysis of CV. In column 4 the percentage of units in each CV group that falls into each receptor class is calculated from the values in column 3. 'Electro sample' in column 6 gives the numbers of neurones used for electrophysiological analysis of all AP variables except AHP amplitude in C cells. The number of C-fibre units with measurable AHPs is indicated in parentheses in column 6. In columns 5 and 7–11 medians are followed by the 25th and 75th percentile values in parentheses. Mann–Whitney *U* tests were carried out between medians of variable of nociceptive (NOCI) and LTM units in each CV group. Kruskall–Wallis with Dunn's *post hoc* tests compared all subgroups of nociceptive units (HTM, MH and MC for C and Aδ groups and MP, other HTM, MH and MC units for Aα/β group) shown in column 2. Finally medians of C cooling units and all C HTM units were compared with the Mann–Whitney *U* test (results shown with a vertical line in column 5). Asterisks show the level of the significance (*P*-values) $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. The absence of asterisks means no significant difference. AP, action potential; AHP, afterhyperpolarization; COOL, cooling units; CV, conduction velocity; E_m, resting membrane potential; HTM, high-threshold mechanoreceptive; LTM, low-threshold mechanoreceptive; MC, mechano-cold; MH, mechano-heat; MRR, maximum rate of rise; MRF, maximum rate of fall; MP; moderate pressure; NOCI, nociceptive; NON-NOCI; non-nociceptive; UNRES, unresponsive.

Nociceptive neurones

Units that failed to respond to the low-intensity stimuli described above but responded either to noxious mechanical stimuli or to both noxious mechanical and noxious heat stimuli were classified as nociceptive. Units classified as 'superficial cutaneous' were those that responded to needle pressure and pinching the superficial skin and lifting it away from the underlying tissue with very fine forceps. These were thought to have receptive terminals in the epidermis or the superficial dermis. Units that did not respond to such manipulations of the

Table 2. Comparison of properties of subgroups of low-threshold mechanoreceptive (LTM) neurones

CV range /receptor class	Total sample (*n*)	% of neurones	CV of total sample (m s^{-1})	Electro sample (*n*)	E_m (−mV)	AP height (mV)	MRR (mV ms^{-1})	MRF (mV ms^{-1})	AHP amplitude (mV)
C LTM units									
LTM			0.44	10	57	61	145	89	3.8
	13	9	(0.34–0.55)***	(10)	(67–47)	(52–79)	(114–597)***	(61–121)**	(2.8–5.1)***
Aδ LTM units									
D hair			3.6		57	65	256	157	7.9
	25	19	(2.8–4.9)	8	(64–51)	(57–68)*	(207–329)**	(121–208)*	(5.2–10.3)*
Aα/β LTM units									
CUT									
F/G			14.6		57	70	394	216	7.3
	183	26	(11.4–19.0)***	68	(64–53)	(62–79)	(298–707)	(158–367)	(4.7–10.4)
RA			17.2		59	75	432	207	6.8
	97	14	(13.5–22.6)	32	(66–56)	(62–85)	(293–533)	(156–276)	(4.0–8.9)
SA			16.8		59	74	364	201	10
	60	9	(13.5–19.5)	18	(69–54)	(63–84)	(271–577)	(175–311)	(7.2–11.0)*
CUT			15.7		58	72	404	213	7.7
	340	46	(12.4–20.0)	118	(65–55)	(62–81)	(293–637)	(164–329)	(4.7–10.5)
MS			18.9		61	71	516	310	8.4
	155	21	(14.4–23.3)***	62	(67–53)	(63–78)	(307–762)	(208–488)***	(5.2–10.3)
Total	495	67		180					

Mann–Whitney *U* tests were used to compare medians for all cutaneous Aα/β units (F/G, SA and RA) grouped together (CUT) with those for MS units and for D hair units. Kruskall–Wallis tests compared all three subgroups of cutaneous (F/G, SA and RA) units as well as the medians for all the CV groups (C and Aδ LTM and Aα/β cutaneous units) and asterisks in the F/G row are for comparisons with RA units. The significance level of differences between C LTM and Aα/β cutaneous units are shown with asterisks in the C LTM row and differences between C LTM and Aδ (D hair) LTM are shown with asterisks on row 2 (indicated with a solid line, column 11). Asterisks indicate levels of significance (see Table 1). CUT, cutaneous; D hair, down hair; F/G, field/guard hair; MS, muscle spindle; RA, rapidly adapting; SA, slowly adapting units. All other abbreviations of variables and the format of the table are as for Table 1.

superficial layers of the skin were classified as 'deep cutaneous' if they required stimulation (squeeze) of a fold of skin including soft dermal tissue. Units that did not respond to these stimuli but whose responses were evoked by squeezing or strong pressure to muscles, joints or deep fascia were classed as 'subcutaneous'.

A-fibre nociceptive neurones in this study included: (1) Aδ-and Aα/β-fibre high-threshold mechanoreceptive (A-HTM) units responding only to noxious mechanical stimuli; (2) A-MC units responding to both noxious mechanical stimuli and cooling; (3) A-mechano-heat (A-MH) units that responded to cutaneous noxious mechanical stimuli and also promptly to a single application of noxious heat. Some Aα/β-fibre nociceptive (HTM) neurones with superficial receptive fields responded to moderate pressure and were defined as 'moderate pressure' (MP) units. These were classed as nociceptive because their adequate stimulus was clearly in the noxious range and they fired more enthusiastically in response to noxious (pin prick or sharp) than to non-noxious stimuli (Burgess & Perl, 1967; for recent review see Djouhri & Lawson, 2004).

C-fibre nociceptive neurones included: (1) units that responded vigorously to both noxious heat and noxious mechanical stimuli, which were classed as C polymodal nociceptive (C-PM) units if they had superficial cutaneous RFs, or mechano-heat (C-MH) units if they had deep cutaneous RFs, (2) MC units that responded to both noxious mechanical and noxious cold stimuli; and (3) HTM units that required strong mechanical stimulation and included both cutaneous units that lacked prompt responses to noxious heat and subcutaneous units that were not tested with heat. Specific C-fibre heat units were not found in this study.

In addition C- or A-fibre units with deep RFs were not tested with thermal noxious stimuli but responded to strong mechanical stimulation and were thus grouped with HTM units.

Unresponsive neurones

Unresponsive units with A- or C-fibres were those for which no RF was found despite an extensive search with the non-noxious, noxious mechanical and thermal stimuli described above. These units have been described in several species (see Discussion).

Non-nociceptive neurones

Non-nociceptive neurones in the present study included low-threshold mechanoreceptive (LTM) units that

Table 3. A summary of electrophysiological differences between different types of DRG neurone

CV range	Receptor class	Total sample (*n*)	CV	Electro sample (*n*)	E_m	AP height	AP OV	AP dur.	AP RT	AP FT	MRR	MRF	AHP dur 80%	AHP amplitude
C	**NOCI**	82	—	47 (43)	—	—	↑	↑↑↑	↑↑↑	↑↑↑	—	—	↑↑	↑↑
	LTM	13	—	10 (10)	—	—					—	—		
	COOL	4	↑	4 (2)	—	—	↓	↓	↓	—	—	—	—	—
	HTM	66		40 (37)	—	—				—	—	—	—	—
Aδ	**NOCI**	77	—	71	—	—	↑↑	↑↑↑	↑	↑↑↑	—	—	↑↑↑	—
	LTM	25	—	8	—	—					—	—		—
All Aα/β	MP(HTM)	26	↑	13	—	—	—	↓	—	↓↓	—	—	↓↓	—
	Other HTM	204		86	—	—	—		—		—	—		—
	MH	4	—	—	—	—	—	—	—	—	—	—	—	—
	MC	3	—	3	—	—	—	—	—	—	—	—	—	—
	NOCI	237	—	102	—	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↓↓↓	↓↓↓	↑↑↑	↑↑
	LTM	495	—	178	—									
Aα/β LTM units	F/G	183		68	—	—	—	↓↓	—	↓↓↓	—	—	↓↓↓	—
	RA	60	↑	32	—	—	—		—		—	—		—
	SA	97	—	18	—	—	—	↓↓	—	↓↓↓	—	—	↓	—
	CUT	340		118	—	—	—		—		—	—		—
	MS	155	↑↑	62	—	—	—	↓↓↓	—	↓↓↓	—	—	↓↓↓	—
Total		732		280										

All statistical tests were non-parametric, comparing medians of all variables listed. Mann–Whitney *U* tests were used to compare the following groups linked with a vertical broken line to the left of column 2: nociceptive neurones with LTM units in each CV group; C-fibre cooling units with C-HTM units, and all Aα/β cutaneous (CUT) afferents with Aα/β MS afferents. Kruskall–Wallis tests with Dunn's *post hoc* test comparing all groups indicated with a solid vertical line to the left of column 2, were used for comparison of subgroups of Aα/β nociceptive units (MP, other HTMs, MH and HC) and of Aα/β cutaneous (CUT) LTM units (F/G, RA and SA). The medians of AP OV, AP Dur, AP RT, AP FT and AHP dur are indicated by horizontal lines in Fig. 3 and the medians of other variables are shown in either Tables 1 and 2 or Fig. 2 (CV). The upwards arrows indicate that the median of the AP variable for that group is greater than that of the compared group whereas the downwards arrows indicate that the median of the variable is smaller than that of the group compared. The number of arrows indicates the level of significance. ↑ or ↓$P < 0.05$; ↑↑ or ↓↓$P < 0.01$; ↑↑↑ or ↓↓↓$P < 0.001$. The absence of arrows means no significant difference. Ampl, amplitude; dur. duration; FT, fall time; OV, overshoot; RT, rise time. All other abbreviations are as for Tables 1 and 2.

responded to non-noxious mechanical stimuli and cooling units.

LTM units were classified according to their responses to non-noxious stimuli. Aα/β LTM units were classed as rapidly adapting (RA) or slowly adapting (SA) units. Among the RA units were the hair follicle afferents that were activated by hair movements. These included guard (G) hair units most of which were well characterized and subdivided into G1 (more rapidly adapting) or G2 (more slowly adapting (Burgess & Perl, 1967). However, others were only partially identified and were not easily distinguishable from field (F) units that were activated effectively by moving a group of hairs (but not single hairs) within the RF. Other types of Aα/β-RA units included sensitive units in glabrous skin (not associated with hairs), units that responded to claw movements, less-sensitive units that required a strong tap but did not respond to sustained light touch stimuli and units that were very sensitive to remote mechanical vibration and followed a tuning fork vibration of 250 Hz, and could be activated by gentle tapping on the surgical frame or the experimental table (possible Pacinian corpuscle units). With the exception of the G/F group, all subgroups of RA units were grouped together as RA units.

Aα/β-RA and SA units were discriminated by their responses to sustained mechanical stimuli (constant pressure applied to the RF with a suprathreshold von Frey hair). SA units showed continuous discharge to constant light pressure with irregular discharge in type I units and regular discharge in type II units. Type II but not type I units were also excited by skin stretch. A group of rapidly conducting Aα/β-fibre units was classified as muscle spindle (MS) afferent units. These often showed ongoing discharges, responded to muscle manipulation, did not have cutaneous RFs and their firing followed a tuning fork vibration of 100–250 Hz. The ongoing discharge in most of these units was probably due to the stretch of the leg muscles. These MS units included groups I and II muscle afferents.

Down hair (D hair) units had Aδ-fibres and were extremely sensitive to slow movement of hairs, to cooling stimuli (described earlier) and skin stretch. C-fibre low-threshold mechanoreceptive (C-LTM) units (also known as C mechanoreceptors) were rare and responded

preferentially to gentle contact moving very slowly across the skin at < 1 mm s^{-1} (but not to rapid hair movements) and sometimes to cooling as previously reported in several species (e.g. Light & Perl, 1993).

Bias in selection of units

Recordings were made from all successfully penetrated Aδ- and C-fibre neurones and from all Aα/β nociceptive including MP units. However, to offset the unintentional bias towards Aα/β-fibre units caused by the greater ease of penetration of large neurones with microelectrodes, many Aα/β-fibre LTM units were rejected during recordings. This resulted in a bias within Aα/β neurones towards those that were nociceptive (see below).

Statistical analysis of data

All neurones regardless of E_m, or other somatic membrane properties were included in CV analyses and determination of proportions of neurones with different sensory properties. For electrophysiological analyses of most AP variables in A-fibre CV group, neurones were included only if they had E_m values more negative than –40 mV, an AHP and an AP overshoot. However, for C-fibre neurones, units were included if they had E_m values more negative than -35 mV regardless of the presence of an AHP (see Djouhri *et al.* 1998). It was previously shown in the guinea-pig that AP variables in C-fibre neurones were similar whether a -30 mV or -40 mV E_m cut-off was used (Djouhri *et al.* 1998). The exceptions to the above were the analyses of AP overshoot and height for which neurones with non-overshooting APs were also included but only if their peak reached a level that was -20 mV or more positive.

In a previous study (Djouhri & Lawson, 2001*b*), we found that electrodes filled with 0.1 M LiCl had much higher resistances than those filled with 1 or 3 M KCl and that this affected AP variables in Aα/β-fibre neurones but not Aδ- or C-fibre neurones. This was also the case in the present study, in which the medians of AP overshoot and AP duration of these Aα/β-fibre units but not Aδ- and C-fibre units were significantly different (not shown) from those of units recorded with KCl-filled electrodes. We therefore excluded all (96) Aα/β-fibre units, recorded with 0.1 M LiCl-filled electrodes from all analyses except CV. Because the values for certain AP variables were not normally distributed, the non-parametric Kruskall–Wallis test with Dunn's *post hoc* test was used to compare medians of three or more unpaired groups instead of the parametric one-way ANOVA and Mann–Whitney *U* tests (Graphpad Prism 4) which were used to compare medians of two groups.

Results

Numbers and percentages of sampled neurones

Intrasomal recordings were made from an overall total of 1022 neurones in L3–L6 DRG neurones (Table 1, column 3). Of these, 463 neurones made up the electrophysiology sample (Table 1, column 6). These were the neurones that fulfilled the electrode criteria (see above) and the normal acceptance criteria of overshooting AP and membrane potential (E_m) of > -40 mV (for A-fibre units) and > -35 mV (for C-fibre units). This sample included 79 C, 98 Aδ and 280 Aα/β-fibre neurones (Table 1, coumn 6) and six neurones that conducted at > 0.8 m s^{-1} and < 1.5 m s^{-1} were classified as C/Aδ units.

Most of the intracellular recordings (97%) were made from the L4 and L5 DRG neurones. RFs of all L4/L5 neurones were located on the hindlimb or flank, but predominantly in the glabrous skin of foot. In contrast, RFs of L6 DRG neurones were limited to the thigh, hip and part of knee and the RFs of the few neurones recorded from L3 were in the knee region. Although RFs were not exhaustively mapped, they were smaller on the foot and digits than on the leg and ankle.

The number and percentages of different subclasses of neurones in each CV group are given in Tables 1–3. The percentages have been calculated from the total sample because this gives a better indication of the real proportion (Table 1, column 4). Units of the total sample that did not fulfil the above acceptance criteria were only used for CV analysis and not for analysis of AP variables shown in Table 1 and Fig. 3. The numbers of different types of units making up the electrophysiology sample are also given in Table 1 (column 6).

Receptive properties of electrophysiologically examined units

Of the electrophysiology sample, 220 units were nociceptive (47 C-, 71 Aδ- and 102 Aα/β-fibre), 196 were LTM (10 C-, eight Aδ- and 178 Aα/β-fibre units), four were cooling (C-fibre) units, and the remaining units were unresponsive (18 C- and 19 Aδ-fibre) (Table 1, column 6). Of the six C/Aδ units, three were HTMs (CV range, 1.03–1.5 m s^{-1}) and three were unresponsive (CV range, 1.0–1.04 m s^{-1}). Unidentified Aα/β-fibre units ($n = 45$) that could have included LTM units with inaccessible RF were excluded.

The high percentage (32%) of Aα/β-fibre units that were nociceptive is an over estimate, caused by intentional rejection of some Aα/β-LTM units during recording. Data from six experiments, with no bias in sampling, reported previously (Djouhri & Lawson, 2004) provided a more accurate value of ~20%.

Nociceptive neurones

Nociceptive units were subdivided according to their RF location (see Methods).

The superficially projecting C-fibre nociceptive units included both HTM and polymodal (MC and MH) units, whereas all the non-superficial units were classified as deep HTM units although they were not tested with thermal noxious stimuli. Of the 47 C-fibre nociceptive units, 29 were deep HTM, 11 superficial/dermal (cutaneous) HTM, five MH and two were MC units.

Of the 71 Aδ-fibre nociceptive units, 32 were deep HTMs, 35 cutaneous (epidermal dermal) HTMs, two MC and two MH units. The cutaneous Aδ nociceptive (HTM, MH and MC) units had punctate superficial RFs (epidermis/dermis/tisuue), whereas the deep HTM units (non-superficial units) with deep RFs were not tested with noxious thermal stimuli. Of the 102 Aα/β-fibre nociceptive neurones, 68 were cutaneous (epidermal/dermal) HTMs, 18 were deep HTMs, 13 were MP and three were MC units.

Unresponsive neurones: a subclass of nociceptors?

In the present study, 34% and 23% of the C- and Aδ-fibre neurones, respectively, were unresponsive (see Table 1). The medians of CVs and AP variables of C- and Aδ-fibre unresponsive neurones were not significantly different from those of C- and Aδ-fibre nociceptors, respectively, with very similar medians and distributions of data in most cases (Table 1). Thus these types of neurone were probably nociceptive units with inaccessible RFs or were very high-threshold nociceptors.

Non-nociceptive neurones

As already stated, non-nociceptive neurones included LTM and cooling units. The latter were only found in the slowly conducting C group, whereas LTM units were found in all CV groups (C, Aδ and Aα/β). However, long T hair (Tylotrich hair) units that were described in the rabbit, cat and guinea-pig (e.g. see Djouhri *et al.* 1998) were not encountered in the present study or in a number of other studies in rat (Lynn & Carpenter, 1982; Handwerker *et al.* 1991; Leem *et al.* 1993), although about 4% of such units were reported in a brief study in rat (Bulka *et al.* 2002). As shown in Table 2, most (69%, 340/495) of the Aα/β-fibre LTM units were cutaneous LTM (RA, SA or F/G) units with superficial or dermal RFs and the remaining (31%, 155/495) were MS units. The proportions of Aδ- (D hairs) and C-fibre LTM units are shown in Tables 1 and 2.

Differences between nociceptive and non-nociceptive neurones

Examples of typical APs recorded from individual neurones with different receptor types are shown in Fig. 1. In each CV group, nociceptive neurones had much broader APs (longer AP and AHP duration) and greater AP overshoots than LTM units.

Conduction velocity

The overall total of 1016 DRG neurones included 151 C-cells, 133 Aδ-cells and 732 Aα/β-cells (see Table 1). Figure 2 displays the distributions of CVs for individual units in each CV group. As can be seen in Fig. 2 and Table 1, for most subgroups of C-fibre neurones the distributions and medians of CV were similar, as previously reported (Gee *et al.* 1999). However, in contrast to that study (Gee *et al.* 1999) and to a study in the guinea-pig (Djouhri *et al.* 1998), the C-fibre cooling units tended to have a faster median CV than the other subgroups (except C MC units) with a significantly faster median CV than the C HTM units (Fig. 2 and Table 1). In the Aδ range, a comparison between the median of D hair and HTM units showed a tendency for the nociceptive units to have faster CVs ($P = 0.06$) and there were too few MH and MC units to be certain of their distribution. In the Aα/β range, the nociceptive had a significantly lower median CV than all the LTM groups (F/G, RA, SA and MS). The MS units had the highest median CV, significantly higher than all cutaneous Aα/β LTM units (RA, SA and G/F units) grouped together (Fig. 2 and Table 2). The F/G units were the slowest conducting Aα/β LTM units, with a significantly lower median CV than both RA and MS units (Fig. 2 and Table 2).

Action potential duration, rise time and fall time

Although there was a considerable overlap between different groups of neurones in AP duration and other AP variables (Fig. 3), LTM units exhibited faster AP kinetics than nociceptive fibres in all CV groups. Indeed C LTM units had much shorter median APs than any other subtype of C-fibre units; significantly shorter than that of C-nociceptive neurones (Fig. 3). C cooling units also had significantly shorter APs than C nociceptive neurones ($P < 0.05$; Mann–Whitney U test).

Both in the Aδ- and Aα/β-fibre range, nociceptive neurones showed a similar pattern with LTM units having significantly narrower spikes than nociceptive neurones (Fig. 3). No significant differences were seen in AP durations between subgroups of C and Aδ nociceptive neurones and were therefore not shown. In contrast, there were differences between subclasses of Aα/β-fibre nociceptive neurones with MP units having significantly shorter AP, FT and AHP durations than other cutaneous HTM units grouped together (Fig. 3*F–H*).

An inverse relationship between somatic AP duration and CV has been reported in several species (Harper & Lawson, 1985; Cameron *et al.* 1986; Rose *et al.* 1986; Ritter & Mendell, 1992; Gee *et al.* 1999) for all types of DRG neurone grouped together. Here we show such plots for

nociceptive and LTM units separately (Fig. 4*A* and *B*). Most noticeably, for A-fibre units there appears to be a stronger relationship for nociceptive than LTM units, that is nociceptive with slower CVs have broader APs (Fig. 4*A*), which is not obviously the case for A-fibre LTM units in Fig. 4*B*. Plotting these data on double log plots (Fig. 4*Aa* and 4*Bb*), however, revealed significant correlations for both nociceptive (Fig. 4*Aa*) and LTM units (Fig. 4*Bb*) but with a greater r^2 for all nociceptive (0.76) than for all LTM units (0.38); the slope of the line for nociceptive units was significantly steeper than that for the LTM units. It is interesting that for all A-fibre units, and for Aδ- and A$\alpha\beta$-fibre units separately there were significant correlations not only for nociceptive but even for LTM units, although with much higher r^2 values for nociceptive units in each case. For example: for all A nociceptive, $r^2 = 0.49$ $P < 0.0001$;

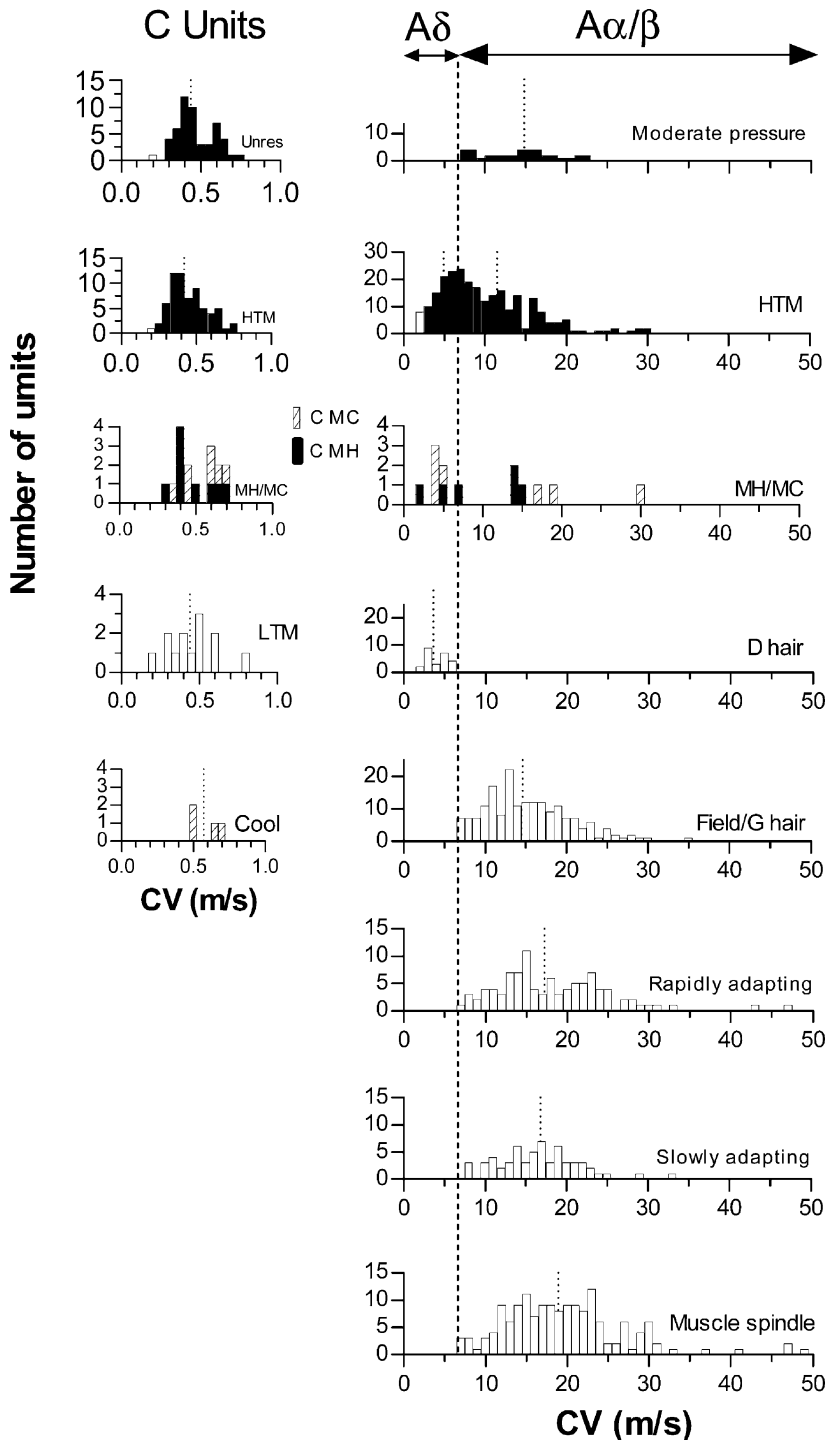

Figure 2. Distributions of CVs for C-, Aδ- and Aα/β-neurones
Subclasses of C- (left), Aδ and Aα/β-fibre (right) DRG neurones are shown. Bin widths are 0.05 m s^{-1} for C-fibre neurones, and 1 m s^{-1} for A-fibre neurones. The short vertical dotted line in each graph indicates the median CV for that subgroup of neurones, whereas the long dotted line over all the right hand graphs indicates the CV borderline (6.5 m s^{-1}) between Aδ- and Aα/β-fibre neurones. Cool, cooling; HTM, high-threshold mechanoreceptor; LTM, low-threshold mechanoreceptor; MC, mechano-cold, MH, mechano-heat; Unres, unresponsive units. Bars in MH and MC graphs are stacked.

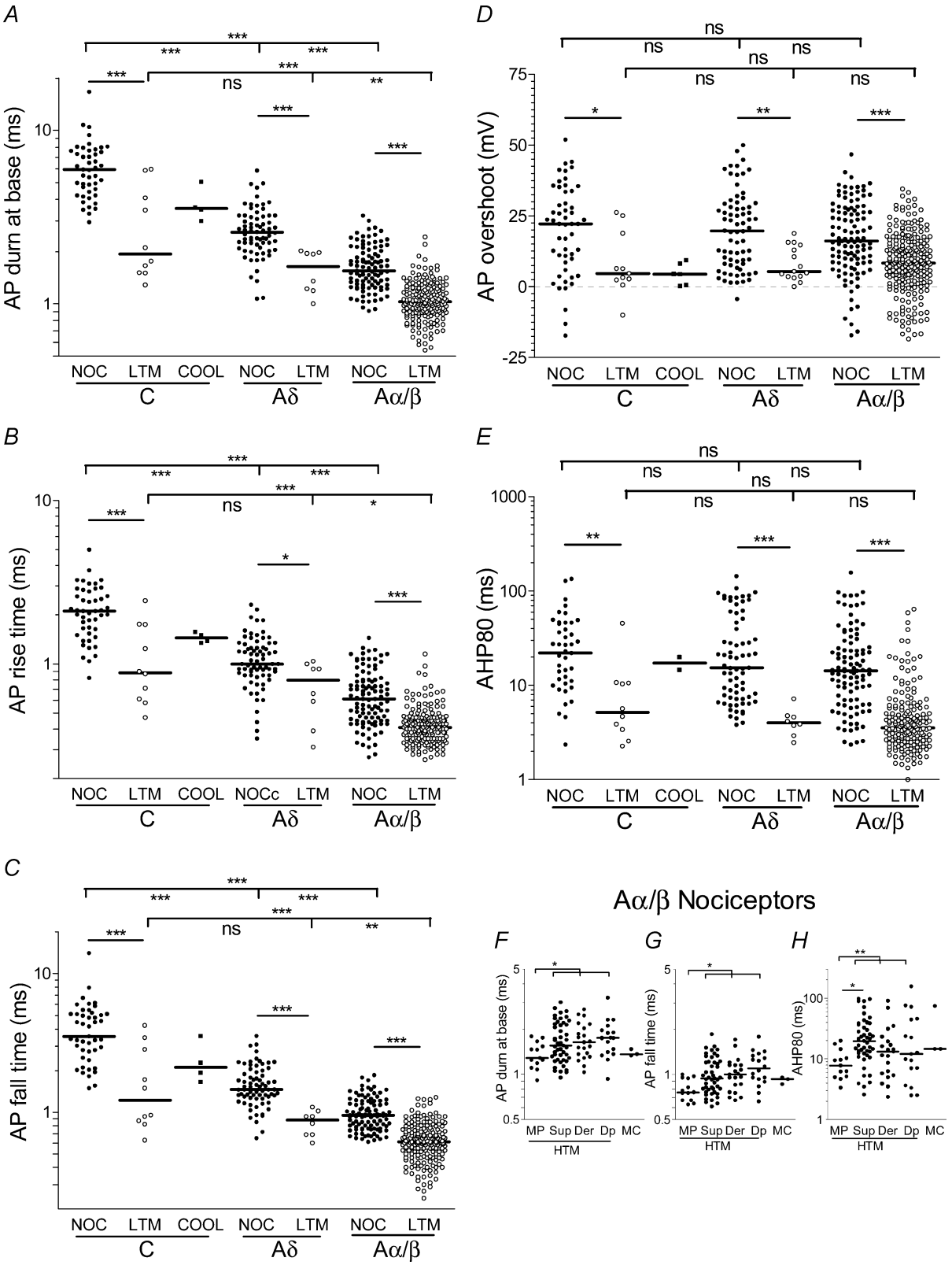

Figure 3. AP variables in nociceptive and other DRG neurones
Scatterplots to show distributions of AP variables with their median (horizontal line) for C-, Aδ- and Aα/β-fibre units (*A–E*). A, AP duration at base; *B*, AP rise time; *C*, AP fall time; *D*, AP overshoot; *E*, AHP duration to 80% recovery (AHP80). *F–H*, selected AP variables for subgroups of Aα/β nociceptors: *F*, AP duration; *G*, AP fall time; *H*, AHP80. Cool, cooling units; LTM, low-threshold mechanoreceptive; MP, moderate pressure; MC, mechano-cold;

and for all A LTM units, $r^2 = 0.16$ and $P < 0.0001$. There were no significant correlations for C LTM or C nociceptive units.

AP rise time (RT) and fall time (FT) were examined separately to determine their relative contributions to the above difference in AP duration between different classes of DRG neurones. In all CV groups (C, Aδ and Aα/β) the medians of both RT and FT duration of LTM units were significantly shorter than those of nociceptive C-, Aδ- and Aα/β-fibre units (Fig. 3 and Table 3). The shorter AP duration in MP than other Aα/β nociceptive units appears to result from the significantly shorter FT (see Fig. 3*G*) because there was no significant difference in the RT.

Action potential overshoot

As shown in Fig. 3 and Table 3, in C-, Aδ- and Aα/β-fibre neurones, nociceptive neurones exhibited significantly larger median AP overshoots than LTM units. Like C LTM, C cooling units had significantly smaller median AP overshoots than C-fibre nociceptive units (Fig. 3*D*). Thus in the rat, LTM units in all CV groups have smaller AP overshoots as previously shown in guinea-pig (Djouhri & Lawson, 2001*b*). Amongst the C-fibre nociceptive neurones, the C MC units had the smallest AP overshoots, and the superficial nociceptive units had the largest (not shown). No significant differences in the median AP overshoot were found between subgroups of Aδ and Aα/β nociceptive neurones (not shown).

Afterhyperpolarization (AHP) duration

In all CV groups, the median AHP duration for LTM units was significantly shorter than that for nociceptive units (Fig. 3). There were no significant differences in median AHP duration between other subclasses of C- or Aδ-neurones subdivided by RF type or location (not shown). However, in the Aα/β-fibre CV group, MP units had significantly smaller median AHP duration than the superficial HTM units and the other cutaneous HTM units grouped together (Sup, Der and Dp; Fig. 3*H*).

Fibre-following frequency

The 80% maximum FFFs of 196 neurones were examined and are shown in Fig. 5. C-fibres had FFFs of < 200 Hz, most Aδ-fibres had FFFs of < 300 Hz, whereas most Aα/β neurones had FFFs of > 300 Hz (maximum 900 Hz). Nociceptive C-fibres had lower FFFs than nociceptive Aδ-fibres, which in turn had lower FFFs than nociceptive Aα/β-fibres (Fig. 5*A*). The median FFF for Aδ LTM units was much slower than that for Aα/β LTM units. Aα/β LTM fibres had a greater median FFF than Aα/β nociceptive fibres. In contrast there was no difference between nociceptive and LTM Aδ-fibres. No significant difference in median FFF existed between subgroups of Aα/β-fibre LTM neurones (MS, SA, RA and G/F) (not shown). C unresponsive units ($n = 5$) had the slowest FFF.

The relationships between FFF and CV in different types of DRG neurones were also examined (Fig. 5*B*). There was a positive correlation in all DRG neurones and for all nociceptive and all LTM units, with much higher r^2 values for nociceptive than for LTM units (Fig. 5*B*) but no significant difference between slopes or between intercepts/elevations of these regression lines. There was also a clear positive correlation for C-fibre nociceptors alone (Fig. 5*B* inset).

The data presented were obtained from 158 rats, although this was not the only type of data collected from these rats. Many were used for dye-injection experiments for subsequent immunocytochemistry in other studies. The mean number of cells per animal was therefore six and a half units, of which about three units (average) met the electrophysiological criteria stated in the Methods.

Causes of the variability of the data include variability between animals (minimized by using only females with similar age and weight) and possibly the variability of temperature near the DRG in the liquid paraffin pool (minimized by including only cells with the temperature near the DRG between 28 and 31°C). To determine whether the overlap in different variables between nociceptive and LTM units (shown with combined data) is also present within individual animals, we compared AP and AHP durations between nociceptive and LTM units in experiments (rats) in which up to 10 Aα/β-fibre neurones were recorded from a single animal. In 10 of such experiments, at least two of the Aα/β-fibre neurones were nociceptive and two were LTM units. In seven of these experiments, there was no overlap in the AP or AHP durations between the two groups, but in three rats there was an overlap in these variables between

MH, mechano-heat; Dp, deep; Der, dermal; Sup, superficial units; NOCI, nociceptive. Asterisks above the graphs indicate the level of statistical significance using the following tests: in *A–E*, the Kruskall–Wallis test with Dunn's *post hoc* test between all groups was used to test between C-, Aδ- and Aα/β-neurones for LTM and nociceptor units; in addition medians for nociceptors and LTM units within each CV group separately were tested with the Mann–Whitney *U* test. In *F–H*, a Kruskall–Wallis test examined differences between superficial, dermal and deep HTM units excluding MP units. Mann–Whitney *U* tests were used to compare MP units with superficial HTM and with all other HTM units (superficial, dermal and deep combined). MH units are not included in these graphs because they did fulfil the electrophysiological acceptance criteria described in the Methods. Significance levels indicated by asterisks as in Table 1 legend.

superficial HTM (including MP units) and LTM units. Thus within individual animals, the only overlap between nociceptive and LTM units in the Aα/β-fibre CV range was in some superficial HTM units. Such comparison could not be made for Aδ-fibre neurones because the Aδ-nociceptive and Aδ-LTM (D hairs) units shown in Fig. 2 and Tables 1–3 were recorded from different animals. For C-fibre neurones, a similar comparison was made only in one experiment in which one C LTM and three C HTM units were recorded from. The C LTM had shorter AP, shorter AHP and a much larger overshoot than the HTM units. Thus the correlation between the somatic AP shape and sensory modality also exists in a given animal and may even be stronger than that shown with the combined data.

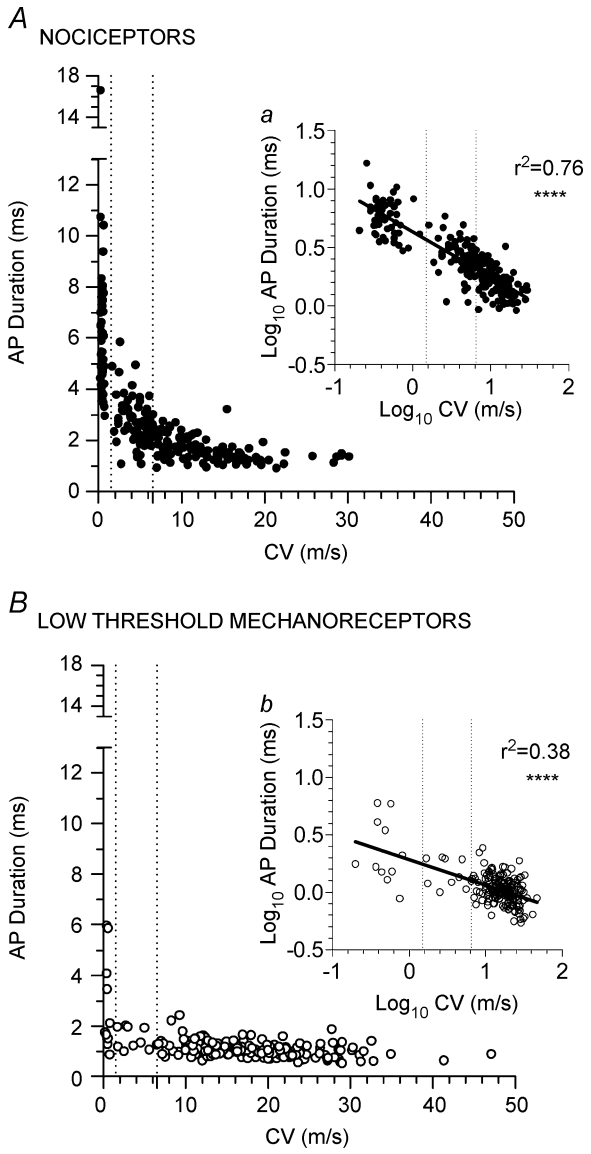

Figure 4. Relationship of CV to AP duration in nociceptive and LTM neurones
Plots for nociceptive (*A*) and LTM (*B*) neurones are shown. A double log plot for each is shown (*Aa* and *Bb*); there was a linear correlation for both nociceptive and LTM units, with a higher r^2 value (see Prism 4 for definition) and significantly steeper slope for nociceptive than LTM units ($P < 0.0001$). Vertical dotted lines indicate the C–Aδ and the Aδ–Aα/β CV borderlines.

Differences between subclasses of Aα/β LTM units

Differences were found between subgroups of Aα/β LTM units. These included CV and AP, FT and AHP duration (Fig. 6 and Tables 2 and 3). Compared with cutaneous Aα/β LTM units (F/G, SA and RA) grouped together, MS units showed significantly larger median CV (Table 2) and smaller median AP, FT and AHP durations (Fig. 6). RA units had significantly longer median AP and AHP durations than those of other cutaneous units (G/F and SA) (Fig. 6). They also had significantly smaller median AHP amplitudes than SA units (Table 2).

Discussion

This is the first comprehensive analysis of sensory and electrophysiological properties of all main subtypes of DRG neurones in the rat. There were clear differences between nociceptive and LTM units in all CV groups and between subgroups of both Aα/β-fibre LTM and nociceptive units. Maximum firing capability differed between DRG neurones in relation to CV.

Types and proportions of different DRG neurones

The sensory receptor types found in the present study were as previously described in several mammalian species including rat (e.g. Lynn & Carpenter, 1982; Leem *et al.* 1993), cat (Burgess & Perl, 1967; Koerber & Mendell, 1992) and guinea-pig (Lawson *et al.* 1997; Djouhri *et al.* 1998). The percentages of those with Aα/β-fibres in the present study may be affected by: (1) the greater ease of making stable intracellular recordings from large somata; (2) the greater likelihood of losing units requiring more intense mechanical stimulation (e.g. deep HTM units); and (3) rejection of many Aα/β LTM neurones during recording. Although Aα/β nociceptive neurones have sometimes been ignored, they do form a substantial group of DRG neurones as indicated by (a) the high percentage of A-fibre nociceptive units that conduct in the Aα/β CV range (75% in the present study and 50–65% in previously published studies, and (b) the percentage (20%) of all Aα/β units that are nociceptive (see Djouhri & Lawson, 2004).

About 9% of our C-fibre afferents were C LTM units, similar to guinea-pig DRG (8%) (Djouhri *et al.* 1998) and rat saphenous nerve afferents (12%) (Lynn & Carpenter, 1982) although a higher percentage (30%) was reported in rat sural nerve (Leem *et al.* 1993).

Unresponsive afferent fibres have been reported in several species including rat (Lynn & Carpenter, 1982; Pini *et al.* 1990; Handwerker *et al.* 1991; Gee *et al.* 1996), guinea-pig (Djouhri *et al.* 1998; Djouhri & Lawson, 1999), cat (Bessou & Perl, 1969) and monkey (Meyer *et al.* 1991). They comprised 34% of C- and 23% of Aδ-fibre units in the present study. In a previous rat study with extracellular recording, about 50% of C- and > 20% of Aδ-fibres were unresponsive (Handwerker *et al.* 1991). Our slightly lower percentages may result from the extensive, destabilizing, search protocol required to class a neurone as unresponsive. As C and Aδ unresponsive units had electrophysiological properties very similar to those of nociceptive neurones both in rat (present study) and guinea-pig (Djouhri *et al.* 1998), most were probably nociceptive with either inaccessible receptive fields or very high thresholds (Djouhri & Lawson, 1999; Xu *et al.* 2000). Inflammation may activate/sensitize some of these units (Schaible & Schmidt, 1988; Meyer *et al.* 1991), but we did not test for this. Although some of these units could be LTM units with inaccessible RFs, their nociceptive-like electrophysiology makes this unlikely.

Differences between nociceptive and non-nociceptive neurones

Conduction velocity. Differences in CV between nociceptive and non-nociceptive neurones and between their subclasses are described in the Results. The lower CV values relative to previously reported values in the rat

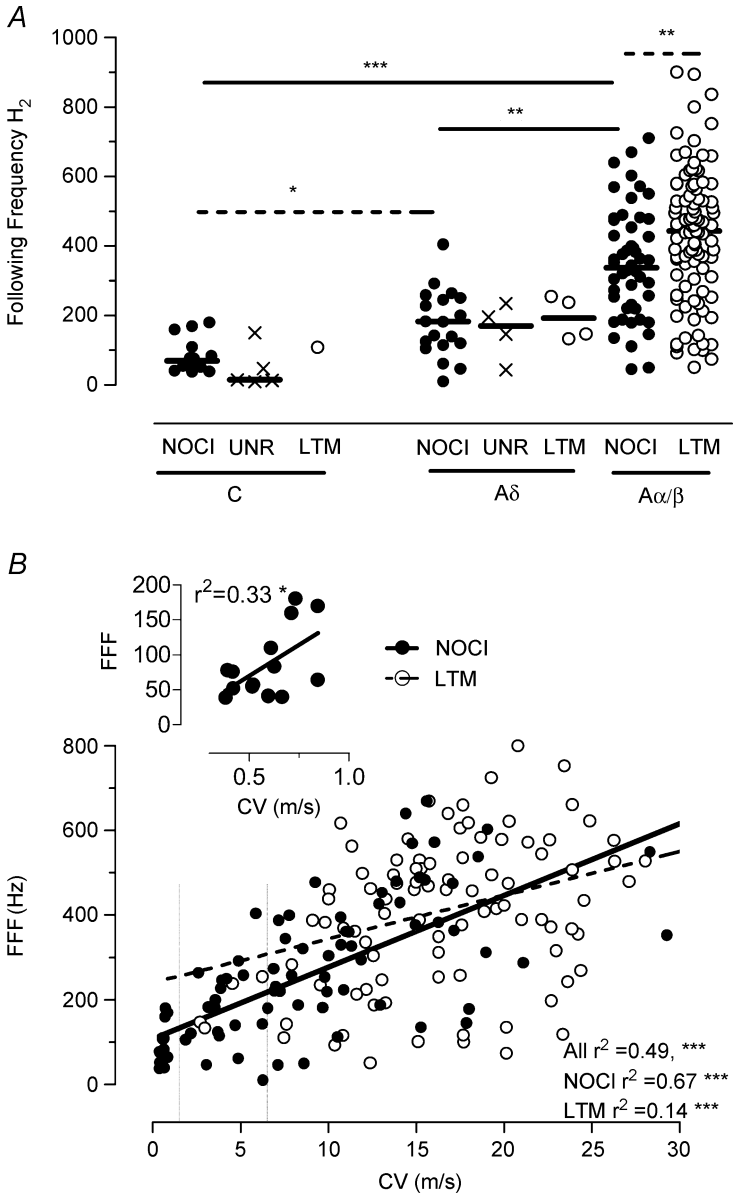

Figure 5. Maximum fibre-following frequencies (FFFs) in different types of neuron
A, the distribution of FFF for nociceptive, unresponsive neurones and LTM units. The Kruskall–Wallis test was used to compare medians of the three nociceptor groups, and significance is shown between groups linked with solid lines. This showed no significant difference between C- and Aδ-nociceptive units, although a Mann–Whitney *U* test did show significance even after Bonferroni's correction was applied (shown above a dotted line). Mann–Whitney *U* tests were carried out between nociceptive and LTM units for Aδ and Aα/β groups, and significance for Aα/β units is indicated above a dotted line. *B*, linear correlation between FFF and CV in nociceptive and LTM populations of DRG neurones. Values for C-fibre nociceptors are plotted separately (inset). Abbreviations and levels of significance for *A* and *B* as in Table 1.

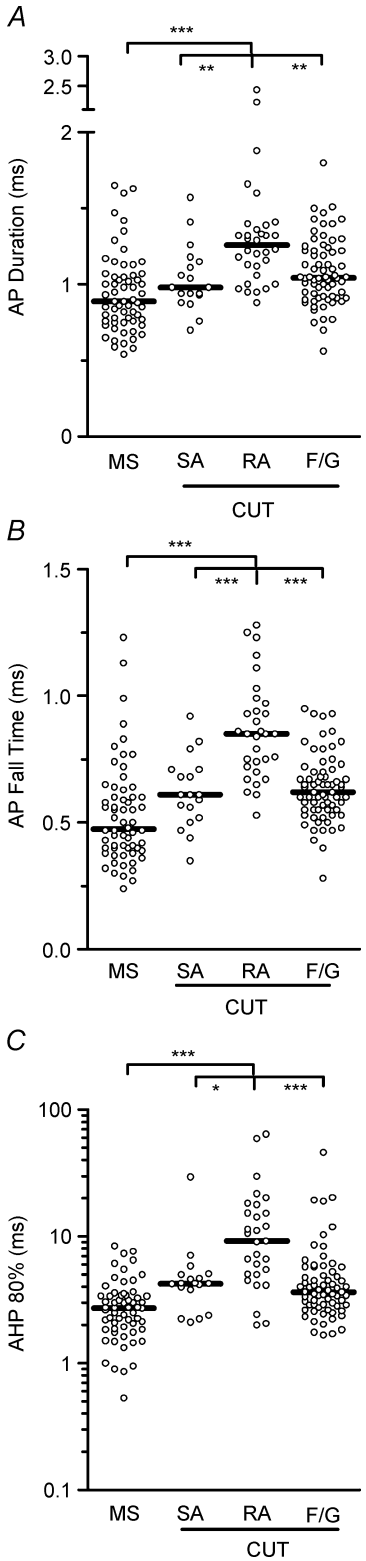

Figure 6. AP variables in subgroups of Aα/β-fibre LTM neurones
For abbreviations see the legend for Table 2. Two tests were carried out on each graph, the non-parametric Kruskall–Wallis test with Dunn's *post hoc* test between all cutaneous LTM units (F/G, SA and RA), and the Mann–Whitney *U* test between MS and all cutaneous LTM units grouped together. For significance levels indicated by asterisks see Table 1 legend.

(Harper & Lawson, 1985; Handwerker *et al.* 1987; Lawson & Waddell, 1991; Leem *et al.* 1993) result from: (1) slower conduction in dorsal root than peripheral nerve fibres (Waddell *et al.* 1989); (2) the relatively low (28–32°C) temperature of the paraffin pool (Franz & Iggo, 1968); and (3) the fact that afferent CVs increase with age beyond the 7 weeks used here (Birren & Wall, 1956; Hopkins & Lambert, 1973). As previously reported (Djouhri & Lawson, 2004), A-fibre nociceptors appears to be a single distribution that peaks close to the Aδ–Aα/β border and has tails in the Aδ and Aα/β CV ranges.

AP variables. LTM units in Aδ and Aα/β CV groups had faster AP and AHP kinetics and smaller AP overshoots than nociceptive neurones, in agreement with previous reports in guinea-pig (Djouhri *et al.* 1998), cat (Rose *et al.* 1986; Koerber *et al.* 1988) and rat (Ritter & Mendell, 1992). As previously reported in guinea-pig (Lawson, 2002), variables that differ consistently between nociceptive and LTM units fall into two categories, those related to CV (AP duration) and those not related to CV (e.g. AP height, AP overshoot, AHP duration) consistent with previous findings in guinea-pig (Djouhri *et al.* 1998). Overall, AP durations are generally longer in the present study at 28–32°C than in studies at 37°C (Harper & Lawson, 1985; McCarthy & Lawson, 1990; Villiere & McLachlan, 1996).

C-fibre LTM units also had faster AP and AHP kinetics and smaller AP overshoots than C-fibre nociceptive units, in agreement with previous studies in guinea-pig (Djouhri *et al.* 1998) and rat (Gee *et al.* 1996), but in contrast to the lack of difference in AP duration between C HTM and C-LTM units in cat (Traub & Mendell, 1988) suggesting a possible species difference.

C-fibre cooling units in the rat have faster AP kinetics and CVs than C HTM units. However in guinea-pig, C-fibre cooling units had similar AP durations and CVs to C-fibre HTM neurones (Djouhri *et al.* 1998). This results from differences in properties of C-fibre cooling units, not C-fibre HTM units between the two species.

Aα/β MP units differed in several ways from other Aα/β HTM units including those with superficial RFs. As well as responding to moderate pressure, they had faster CVs, and faster AP and AHP kinetics, such that all these properties were intermediate between those of Aα/β nociceptive and LTM units. As MP units fire more rapidly in response to clearly noxious mechanical stimuli, they are classed as nociceptive although, interestingly, they express much less trkA, the high affinity receptor of nerve growth factor (that contributes to the development and maintenance of nociceptive neurones), than other HTM units (Fang *et al.* in press). It may be important to have units with RFs in the superficial skin that encode intensity in the range between LTM and HTM units, as an early trigger of a withdrawal reflex, perhaps even

before a fully noxious/damaging stimulus level has been reached, may have survival advantages. A small lowering of threshold, and/or increased firing rate in MP units at lower stimulus intensities, could contribute to pain or unpleasant sensations in response to low-intensity cutaneous stimuli (Djouhri & Lawson, 2004). While there is evidence for Aα/β-fibres contributing to tactile allodynia after nerve or tissue injury, this has sometimes been assumed to involve LTM units and a possible contribution from Aα/β nociceptive neurones has not been examined.

Fibre-following frequency. That C-fibres were capable of following lower frequency stimulus trains than Aδ- and Aα/β-fibre neurones, is consistent with previous observations *in vitro* in rat (Waddell & Lawson, 1990) and *in vivo* in guinea-pig (Djouhri *et al.* 2001). A possible interpretation for the novel findings that the regression lines of CV *versus* FFF for nociceptive and LTM units are similar, is that fibre diameter/CV may have a greater influence on FFF than the types of ion channel expressed selectively in nociceptors or LTM units.

Differences between subtypes of Aα/β-fibre LTM units

Some electrophysiological difference between subgroups of Aα/β-fibre LTM units resemble those in guinea-pig (Djouhri *et al.* 1998) with MS units having faster AP/AHP kinetics and median CVs than most cutaneous LTM units, and with RA units having slower AP/AHP kinetics than other cutaneous Aα/β LTM units. The fast AP kinetics of MS afferent units are consistent with their capabilities of sustained and/or very rapid firing, and the slow kinetics in RA units especially of the AHP may help limit their firing to a few APs.

Ionic mechanisms that may underlie differences in AP variables

As all three major groups of ion channels (Na^+, Ca^{2+} and K^+) are involved in determination of AP configuration, differences in their expression and/or activation/kinetics must underlie the differences in AP configuration. Na^+ channels are likely to have a major influence on AP rise time and therefore on AP duration and CV. For example, the greater AP overshoot in nociceptive neurones is likely to result from the high TTX-R Nav1.8-related Na^+ current (Herzog *et al.* 2001; Djouhri *et al.* 2003*a*), which contributes most of the inward Na^+ current during the AP rising phase in small DRG neurones (Decosterd *et al.* 2002). Nav1.8 is one of three Na^+ channels (Nav1.7, Nav1.8 and Nav1.9) expressed more highly in nociceptive units, and that are correlated with AP duration in some types of nociceptive neurone (Fang *et al.* 2002; Djouhri *et al.* 2003*b*). Therefore these three Na^+ channels may all contribute to AP profiles in nociceptive neurones. Differences in expression and/or activation of both voltage-gated and Ca^{2+}-activated K^+ channels are likely to contribute to longer AHPs in nociceptors (see Sah, 1996; Vogalis *et al.* 2002). Differential expression and/or activation of other currents that suppress or delay spike generation (e.g. I_A, fast transient K^+ currents, or I_H, hyperpolarization-activated currents) may also contribute to the differences in FFF seen between different types of DRG neurones.

In conclusion, in the rat as in the guinea-pig (Djouhri *et al.* 1998), nociceptive DRG neurones *in vivo* are likely to have a large AP overshoot, coupled with a long AHP and long AP duration. The AP long duration is especially marked in slowly conducting nociceptive neurones (likely to be small) while the other two variables are not dependent on CV. Using these criteria to determine nociceptor identity should prove effective for neurones acutely *in vitro* (hours) as long as effects of temperature are considered, but may prove less useful after longer times (days) as these variables may change with time elapsed after axotomy and/or dissociation.

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