Receptive Properties of Mouse Sensory Neurons Innervating Hairy Skin

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Koltzenburg, Martin, Cheryl L. Stucky, and Gary R. Lewin. Receptive properties of mouse sensory neurons innervating hairy skin. J. Neurophysiol. 78: 1841–1850, 1997. Using an in vitro nerve skin preparation and controlled mechanical or thermal stimuli, we analyzed the receptive properties of 277 mechanosensitive single primary afferents with myelinated (n = 251) or unmyelinated (n = 26) axons innervating the hairy skin in adult or 2-wk-old mice. Afferents were recorded from small filaments of either sural or saphenous nerves in an outbred mouse strain or in the inbred Balb/c strain. On the basis of their receptive properties and conduction velocity, several receptor types could be distinguished. In adult animals (>6 wk old), 54% of the large myelinated fibers (Aβ, n = 83) showed rapidly adapting (RA) discharges to constant force stimuli and probably innervated hair follicles, whereas 46% displayed a slowly adapting (SA) response and probably innervated Merkel cells in touch domes. Among thin myelinated fibers (Aδ, n = 91), 34% were sensitive D hair receptors and 66% were high-threshold mecanoreceptors (AM fibers). Unmyelinated fibers had high mechanical thresholds and nociceptive functions. All receptor types had characteristic stimulus-response functions to suprathreshold force stimuli. Noxious heat stimuli (15-s ramp from 32 to 47°C measured at the corium side of the skin) excited 26% (5 of 19) of AM fibers with a threshold of 42.5 ± 1.4°C (mean ± SE) and an average discharge of 15.8 ± 9.7 action potentials, and 41% (7 of 17) C fibers with a mean threshold of 37.6 ± 1.9°C and an average discharge of 22.0 ± 6.0 action potentials. Noxious cold stimuli activated 2 of 10 AM fibers and 3 of 10 C fibers. One of 10 C units responded to both heat and cold stimuli. All types of afferent fibers present in adult mice could readily be recognized in mice at postnatal day 14. However, fibers had reduced conduction velocities and the stimulus-response function to mechanical stimuli was more shallow in all fibers except for the D hairs. In juvenile mice, 22% of RA units also displayed an SA response at high stimulus intensities; these units were termed RA/SA units. We conclude that all types of cutaneous afferent fibers are already committed to their phenotype 2 wk after birth but undergo some maturation over the following weeks. This preparation has great potential for the study of transgenic mice with targeted mutations of genes that code factors that are involved in the specification of sensory neuron phenotypes.

INTRODUCTION

The receptive properties of primary afferent neurons with myelinated (A fibers) or unmyelinated (C fibers) axons have been extensively studied in mammalian preparations (Perl 1992). These studies have shown that primary afferent fibers can be grouped into distinct functional subpopulations on the basis of their receptive field properties. These functional properties can also be linked to characteristic neurochemical, biophysical, and anatomic features (Koerber and Mendell 1992; Lawson 1992; Willis and Coggeshall 1991). The distinction between the different classes of primary afferent neurons is most elegantly illustrated by the fact that humans experience distinct elementary sensations such as vibration, pressure, or pain when axons of functionally different subpopulations of sensory neurons are selectively stimulated with the use of intraneural electrical microstimulation (Torebjörk et al. 1987). Work in animals has demonstrated that this specificity is probably the result of modality-specific anatomic and functional connections that these afferent fibers make in the CNS (Brown 1981; Koerber and Mendell 1992; Vickery et al. 1994). It is therefore of general interest to understand the factors that control the properties of primary afferent neurons and lead to the specification of their peripheral and central terminations.

During normal development, the number of neurons expressing functionally distinct receptive properties appears to be tightly regulated, and the accessibility of secreted proteins, including members of the neurotrophin family, have been implicated in this process (Lewin 1996). One can manipulate the levels of such factors in animals by using blocking antibodies against endogenous factors (Johnson et al. 1980; Ritter et al. 1991; Rohrer et al. 1988) or by administration of excess amounts of these factors (Lewin et al. 1993). However, it also clear that the specification of primary afferent properties must be the result of interactions between many different gene products during development, notably extracellular matrix molecules, or of different combinations of transcription factors (Akopian et al. 1996); and these may not be easily amenable to similar experimental manipulations. One further way of manipulating the levels of such factors is through the use of gene targeting technology. This technology is most highly developed for mice, where the number of gene products that have been deleted or added in vivo was recently estimated at being on the order of several hundred (Brandon et al. 1995). Many of the these mice show profound abnormalities involving sensory neurons (Albers et al. 1994; Snider 1994). It is therefore increasingly important to know the normal receptive properties of sensory neurons in mice to be able to interpret the results of such studies. Although receptive properties have been extensively studied in other rodents, notably rat (Fleischer et al. 1983; Kress et al. 1992; Leem et al. 1993; Lewin and McMahon 1991; Lewin and Mendell 1994; Lynn and Carpenter 1982; Reeh 1986), little is known about the functional properties of sensory neurons in normal mice, which are not easily accessible to conventional neurophysiological
recordings in vivo (Scadding 1981). Here we have systematically examined the receptive properties of mouse cutaneous receptors in situ by adapting an in vitro skin nerve preparation that circumvents many of the technical problems encountered in conventional in vivo studies (Kress et al. 1992; Reeh 1986). Stable recordings were obtained from animals as young as postnatal day (P) 14; therefore this preparation has great potential in the study of the factors that lead to the specification and maintenance of the sensory neuron phenotype.

METHOnS

Animals

Mice of either sex weighing 7–38 g were used in this study. They were either from an inbred Balb/c strain purchased from Charles River (Sulzdorf, Germany) or from an outbred strain derived from a genetic background of C57BL/6, NMIR, and 129 raised in the Max-Planck-Institute for Psychiatry (Martinsried, Germany). Animals were killed by CO₂ inhalation, and after the hair was clipped, the skin of the hindlimb with either saphenous or saphenous nerve attached was removed. In one series of experiments, recordings were obtained from the saphenous nerves of outbred animals aged 5–32 wk or P14–16. In another series of experiments, recordings were obtained from the sural nerves of Balb/c mice. Some of the recorded units have also been included in a previous publication (Airaksinen et al. 1996).

Skin-nerve preparation

The mouse skin-nerve in vitro preparation was modified from a rat preparation that has been described in detail previously and in which the properties of afferent fibers in vitro are essentially the same as in vivo (Kress et al. 1992; Reeh 1986). The nerve was dissected free to the lumbar sacral plexus to ensure a sufficient length of nerve for recording. After dissection, the preparation was placed “inside-up” in an organ bath to facilitate oxygenation through the corium side of the skin (Fig. 1) and the preparation was superfused (15 ml/min) with an oxygen-saturated modified synthetic interstitial fluid solution containing (in mM) 123 NaCl, 3.5 KCl, 0.7 MgSO₄, 1.7 NaH₂PO₄, 2.0 CaCl₂, 9.5 sodium gluconate, 0.75 glucose, 7.5 sucrose, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), pH 7.4 ± 0.05 (SE), temperature 32 ± 0.5°C.

Recording technique

With the use of sharpened watchmakers’ forceps, filaments were teased from the desheathed nerve and single sensory neurons were recorded extracellularly with a low-noise differential amplifier. Receptive fields of primary afferent fibers were identified with a mechanical search stimulus (manual probing of the skin with a glass rod) that is known to activate >90% of rat cutaneous afferent fibers in this preparation (Kress et al. 1992). Only units with a signal-to-noise ratio of >3 were used for further analysis. The conduction velocity of each axon was determined by electrically stimulating the receptive field with supramaximal square-wave pulses (duration 0.1–1.0 ms, interstimulus interval 1–5 s) with a Teflon-coated steel needle electrode with an uninsulated tip diameter of 10 μm. Recordings of compound action potentials were used in pilot experiments to determine the conduction velocity of the different fiber groups in the nerves. In agreement with single-unit recordings in the rat, a cutoff of 1.2 m/s was used to distinguish between myelinated and unmyelinated fibers (Kress et al. 1992). Fibers conducting more quickly than 10.0 m/s were considered to be units with large myelinated (Aβ) axons, whereas units below this value have thin myelinated (Aδ) axons. Because the single-fiber recording technique is biased against small axons, the relative proportion of different afferent types that were sampled was expressed for Aβ-, Aδ-, and C fibers separately. The identity of the action potentials evoked by electrical or adequate natural stimulation was determined on the basis of the shape of the action potentials and whether the electrical stimulation of a skin region excited only a single unit. In addition, a marking procedure was employed for C fibers as described previously (Kress et al. 1992) if there was any doubt about the unitary identity of the response. The latency of a C fiber with electrical stimulation in the receptive field or in the nerve is generally stable at low interstimulus frequencies. However, this latency typically increases if the unit is excited (e.g., by adequate mechanical stimulation) between the regular electrical stimulation was determined on the basis of the shape of the action potentials and whether the electrical stimulation of a skin region excited only a single unit. In addition, a marking procedure was employed for C fibers as described previously (Kress et. 1992) if there was any doubt about the unitary identity of the response. The latency of a C fiber with electrical stimulation in the receptive field or in the nerve is generally stable at low interstimulus frequencies. However, this latency typically increases if the unit is excited (e.g., by adequate mechanical stimulation) between the regular electrical stimulation (Iggo 1958; Schmelz et al. 1995). After determination of the conduction velocity, fibers were subjected to a standard protocol of adequate stimuli consisting of a sequence of mechanical stimuli. Fibers with potential nociceptive properties [high-threshold mechanosensitive nociceptive A (AM) fibers and C fibers] could then be studied with a noxious cold stimulus followed by a noxious heat stimulus.

Mechanical stimulation

The mechanical threshold of each unit was determined with calibrated von Frey monofilaments with uniform tip diameter of 0.8 mm. The lowest von Frey filament used in this study exerted a bending force of 1 mN, because smaller monofilaments were often incapable of reliably penetrating the surface tension of the bath. Constant force stimuli were applied with the use of a feedback-controlled right cylindrical probe with a tip diameter of 0.8 mm placed perpendicularly onto the most sensitive spot of the receptive field. Each stimulus began with an adaptation period of 5 s at a force of 1 mN. In very sensitive units, this minimal force evoked a discharge that in most cases disappeared within 5 s or settled at a low level of activity. Then the force rose within 200 ms to a preset force plateau that varied between 5 and 300 mN. After 10 s, force returned to the adaptation force of 1 mN for 5 s before the probe was lifted off the tissue. In pilot experiments, we determined that this stimulus configuration was suitable for clearly differentiating the adaptation properties of the different fibers. Stimuli were delivered in ascending order, one every minute. Longer interslot intervals might have been advantageous to completely avoid interstimulus interaction. However, in initial experiments we determined that desensitization was most noticeable when a high-force stimulus was followed by a very low-intensity
Thermal stimulation

In a subpopulation of fibers, thermal stimuli were also tested. After the mechanical stimuli, the receptive field of the neuron was isolated with a small self-sealing metal ring (6 mm diam). The bath solution within the ring was manually removed with a syringe and a thermocouple was gently applied to measure intracutaneous temperature.

Cold stimuli were delivered by giving a 10-ml bolus injection of ice-cold synthetic interstitial fluid solution, which resulted in a temperature nadir of 4–6°C, after which the temperature returned passively to baseline within 1–2 min. Care was taken not to apply the force of the injection stream directly to the receptive field to avoid nonspecific excitation of very mechanically sensitive units. A true cold discharge was scored when the unit discharged at least three action potentials during the drop of temperature until the nadir was reached and control injections of fluid at 32°C did not evoke a discharge. Because the application of cold was the only stimulus that was not controlled by a feedback system, the responses are reported only qualitatively.

After skin temperature had returned to baseline and the fluid within the ring had been removed again, a standard heat stimulus was delivered to the epidermal side of the preparation through the translucent bottom of the organ bath. A halogen bulb was focused onto the receptive field and skin temperature rose linearly within 15 s from 32 to 47°C at a rate of 1°C/s. The heat threshold of units was either defined as the temperature that elicited the second spike of the response or as the temperature that evoked an instantaneous frequency >1.0 imp/s. Both measures gave identical average results (see below).

Data analysis and statistical tests

All data were collected with the use of custom-made data acquisition software running on a PC and action potentials were subsequently analyzed with a template-matching program (Forster and Handwerker 1990). Further quantitative analysis of the recorded neural responses was carried out with custom-designed software. For construction of the stimulus-response functions for mechanical stimulation, all action potentials were counted in a time period of 11 s after the onset of the force stimulus. This time window was chosen because it contained all the spikes during the rise time, plateau, and release of the stimulus. Mean mechanical and heat responses of the discharge were also plotted by averaging the responses in 1-s bins for all fibers tested. The heat response was also displayed as a scatter plot of the running (adjacent 3-point) average of this histogram of the discharge for the time period of the temperature increase of the standard heat ramp. A linear regression analysis was then used to correlate skin temperature with the neural activity (Koltzenburg et al. 1992). The total number of action potentials was also counted for a period of 16 s after the onset of the stimulus.

All values are given as means ± SE except for the von Frey hair thresholds, which are given as median and the interquartile range between the first and third quartiles. Appropriate statistical tests (unpaired 2-sided Student’s t-test, Mann-Whitney U test, and χ² test) were used after fulfillment of the necessary prerequisites with the use of the Statistica software package of Statsoft.

RESULTS

Sample of units

A total of 277 single units was recorded in the present investigations. Of those, 217 fibers were studied in adult animals and 60 in juvenile mice. Except for 26 units, all fibers conducted more quickly than 1.2 m/s and were therefore considered to be myelinated fibers. In adult animals, 111 units were obtained from the saphenous nerve and 106 from the sural nerve. All recordings from juvenile animals were obtained from the saphenous nerve. In one set of survey experiments, an attempt was made to classify each unit with a sufficient signal-to-noise ratio that was present in randomly selected filaments that contained only few functional units (usually ≤3). On this basis of 234 units (111 from adult saphenous nerve, 63 from adult sural nerve, and 60 from juvenile saphenous nerve) a remarkable consistency of the proportions was found between different nerves, different strains, and different ages (Table 1; vide infra). In another set of experiments on adult animals, 43 myelinated or unmyelinated nociceptors were selected for further detailed investigations of their stimulus-response functions to standard heat or mechanical stimuli. Because there was no significant difference in the distribution of different receptor subtypes, the conduction velocity, or the mechanical or thermal thresholds, the data were pooled (Table 1; Figs. 4 and 6).

Functional types of mechanosensitive units

Both myelinated and unmyelinated mechanosensitive fibers generally displayed no ongoing activity in the absence of intentional stimuli. In adults, four distinct receptor types with myelinated axons were distinguished on the basis of their conduction velocity, their adaptation properties, and their conduction velocity.

<table>
<thead>
<tr>
<th>Type</th>
<th>Proportion</th>
<th>Conduction Velocity, m/s</th>
<th>von Frey Hair Threshold, mN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saphenous nerve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(adult outbred mice)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>52% (28/54)</td>
<td>13.6 ± 0.4</td>
<td>1.0 (0.2)</td>
</tr>
<tr>
<td>SA</td>
<td>48% (26/54)</td>
<td>15.5 ± 0.5</td>
<td>1.0 (0.0)</td>
</tr>
<tr>
<td>DH</td>
<td>35% (20/57)</td>
<td>4.8 ± 0.4</td>
<td>1.0 (0.0)</td>
</tr>
<tr>
<td>AM</td>
<td>65% (37/57)</td>
<td>7.0 ± 0.5</td>
<td>5.6 (5.2)</td>
</tr>
<tr>
<td>Sural nerve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(adult Balb/c mice)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>59% (17/29)</td>
<td>13.5 ± 1.3</td>
<td>1.0 (1.0)</td>
</tr>
<tr>
<td>SA</td>
<td>41% (12/29)</td>
<td>1.2 ± 0.5</td>
<td>1.0 (0.5)</td>
</tr>
<tr>
<td>DH</td>
<td>32% (11/34)</td>
<td>5.3 ± 0.8</td>
<td>1.0 (0.0)</td>
</tr>
<tr>
<td>AM</td>
<td>68% (23/34)</td>
<td>5.7 ± 0.8</td>
<td>4.0 (1.6)</td>
</tr>
<tr>
<td>Saphenous nerve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P14–16 outbred mice)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>RA</td>
<td>30% (8/27)</td>
<td>9.7 ± 0.7</td>
<td>1.0 (0.4)</td>
</tr>
<tr>
<td>RA/SA</td>
<td>22% (6/27)</td>
<td>10.1 ± 0.9</td>
<td>1.0 (0.0)</td>
</tr>
<tr>
<td>SA</td>
<td>48% (13/27)</td>
<td>10.6 ± 0.6</td>
<td>1.4 (3.0)</td>
</tr>
<tr>
<td>DH</td>
<td>45% (15/33)</td>
<td>3.8 ± 0.5</td>
<td>1.0 (0.0)</td>
</tr>
<tr>
<td>AM</td>
<td>55% (18/33)</td>
<td>3.2 ± 0.4</td>
<td>5.6 (6.0)</td>
</tr>
</tbody>
</table>

Values for conduction velocity are means ± SE; those for von Frey hair thresholds are median and interquartile range. Except for the conduction velocity of slowly adapting (SA) fibers, there was no significant difference between sural or saphenous nerve for proportion of different receptor types (P always >0.2, χ² test), the conduction velocity (P always >0.2, t-test), and the von Frey thresholds (P always >0.4, U test). RA, rapidly adapting; DH, D hair; AM, high-threshold mechanosensitive nociceptive A fiber; P, postnatal day.
the size of the receptive field (Fig. 1). Among the units with thin myelinated (Aδ) axons, two receptor types were found, namely D hair (DH) receptors or AM fibers. The receptive fields of both types of units covered several square millimeters and many had sensitive spots where mechanical stimulation was more effective than in the rest of the receptive field. DH units had extremely low mechanical thresholds and always responded to von Frey hairs with a bending force of 1.0 mN. They showed a brisk rapidly adapting (RA) discharge at the onset and offset of a supramaximal constant force stimulus. AM units had thresholds that usually exceeded 1.0 mN and all had a slowly adapting (SA) discharge to suprathreshold constant force stimuli. These fibers are also known as high-threshold mechanoreceptors (Lewin et al. 1992; Perl 1992). The detailed morphological structure of the receptive terminals of DH receptors is not known, but it is likely that they correspond to fibers that innervate hair follicles (Light and Perl 1993; Willis and Coggeshall 1991). Many AM units have nonspecialized terminals that end at the dermal-epidermal border (Kruger et al. 1981).

Fibers conducting more quickly than 10 m/s (Aβ-fibers) had either RA or SA properties to a constant force stimulus. Many RA fibers probably innervate the hair follicles and probably correspond to G hair units (Light and Perl 1993), whereas most SA units supply the Merkel cell complexes in the touch domes (Haarscheiben) (Airaksinen et al. 1996; Iggo and Andres 1982; Willis and Coggeshall 1991). Because of the inside-up mounting of the skin and the small size of touch domes (which can barely be discerned in living mice), a more detailed correlation between the response properties and the morphology of the skin appendages could not be obtained. Moreover, a clear distinction of different types of hairs is usually not possible in the distal hindlimb of rodents (Lynn and Carpenter 1982). Most of the fibers had small, punctate receptive fields and the majority responded readily to stimulation with a von Frey hair exerting a bending force of 1.0 mN.

In juvenile animals, a third type was regularly found among the Aβ-fibers that probably represents an intermediate, not fully differentiated type of receptor (Fig. 2). This unit was classified as an RA/SA unit because it responded over a range of low stimulus intensities with an RA discharge but displayed an SA discharge at higher forces. One could argue that this type was an SA unit that was not optimally stimulated and therefore discharged only few action potentials at lower intensities. However, we think that this is an unlikely explanation. First, when the location of the probe was changed, the response remained qualitatively the same. Second, the adaptation properties of true RA or SA fibers remained the same throughout a range of suprathreshold stimuli in both juvenile and mature animals (Fig. 3). Because the proportion of SA fibers among the Aβ-units was not significantly different between adult and juvenile animals (Table 1), it is likely that the RA/SA fibers constitute an immature population of fibers that is destined to become RA fibers as the animal ages.

In adult animals, a total of 26 units with unmyelinated axons was also investigated in the present study and a representative example is shown in Fig. 4. Probably all of the units had nociceptive functions and their properties resembled in many aspects the properties of thin myelinated (AM) nociceptors. C fibers generally responded with an SA discharge to constant force stimuli and were often excited by nonme-
Conduction velocities of mechanoreceptors

Figure 5 shows the distribution of conduction velocities found for functionally distinct receptors with myelinated axons (see also Table 1 for mean values). In adult animals, AM units spanned a wide range, with the fastest unit conducting just above 15 m/s, but ≥80% of the afferent fibers had a conduction velocity <10 m/s. It seems unlikely that the fibers conducting more quickly than 10 m/s were insensitive SA mechanoreceptors because of the different response profile to suprathreshold stimuli. DH receptors had the lowest mean conduction velocity and all units fell below 10 m/s. In contrast to the high-threshold mechanoreceptors, the distribution of the conduction velocity of DH units was much narrower. Virtually all SA or RA afferent fibers conducted more quickly than 10 m/s. These results further confirm the validity of the cutoff values between small- and large-diameter afferent fibers with myelinated axons as defined from preliminary recordings of compound action potentials.

The distinction of the conduction velocities between different receptors was already present in animals that were only 2 wk old. Because myelination of peripheral nerves is not complete at this stage, the average conduction velocities were ~2–4 m/s slower. Also, the distribution for both AM and DH units is skewed toward lower values, which could mean that some of the fibers are still unmyelinated and only become myelinated as the animal ages. Indeed, the total number of myelinated axons observed at the light microscopic level is 15% less in P14 mice compared with mature adults (unpublished observations). A cutoff value that separated prospective Aβ- and Aδ-units was 7.0 m/s at the end of the second postnatal week. In the adult animal, the mean conduction velocity of the unmyelinated mechanosensitive afferent fibers was 0.69 ± 0.05 m/s, and >90% of these units had conduction velocities <1.0 m/s.

Quantitative analysis of mechanical sensitivity

Measurements of the mechanical thresholds with the use of von Frey hairs showed that mechanoreceptors were already mature for most myelinated fibers at P14 (Table 1; Fig. 6). In both juvenile and adult mice, DH receptors were always excited by 1.0 mN at both ages. The distribution of thresholds for AM and RA (and RA/SA units) fibers in juvenile mice was indistinguishable from that found in adult mice. However, SA afferent fibers appeared to have higher thresholds at P14 compared with adults (Fig. 6).

A feedback-controlled mechanical stimulator was used to construct stimulus-response functions at suprathreshold stimulus intensities for the different receptor types in both juvenile and adult animals (Fig. 7). This analysis revealed that the typical discharge pattern of each functional type of unit was already distinguishable at P14. Among the units with rapidly adapting properties (RA, DH), stimulus intensities <40 mN elicited the maximal excitation, as would be expected for units that signal innocuous hair movements. Although the coding range was very narrow in RA or DH units at both ages studied, there was a profound difference in the number of action potentials evoked by the force stimuli. Among the slowly adapting receptors (SA, AM) there was a characteristic difference in the coding properties in the adult. In agreement with the different von Frey thresholds, SA fibers discharged on average >3 imp/s at the lowest stimulus intensity. By contrast, in AM fibers the mean activity was minimal below a force of 40 mN. In adult mice, SA fibers increased their discharge frequency up to 40 mN and then reached a plateau, whereas AM fibers displayed a monotonically rising stimulus-response function throughout the stimulus range tested. Thus SA fibers encode the magnitude of a force stimulus in a range <40 mN and AM fibers encode stimulus force in a range >20–40 mN. Moreover, during suprathreshold stimulation the SA fibers displayed a typical initial burst of activity, whereas AM fibers did not and often increased their discharge frequency during the stimulus plateau (Fig. 1). The different working ranges of the two populations are consistent with the fact that SA and AM fibers subserve nonnociceptive and nociceptive functions, respectively.

The threshold as well as the responses to suprathreshold force stimuli of unmyelinated were both qualitatively and quantitatively very similar to those of thin myelinated nociceptors (Fig. 8). C fibers had a median von Frey hair threshold of 5.6 mN (interquartile range 2.8).

Qualitatively, the stimulus-response functions of mechanoreceptors in juvenile animals were very similar to those in adults. This is consistent with the view that virtually all afferent fibers are functionally committed to a certain receptive function by the end of the second postnatal week. However, the response of the units was not as brisk as in the adults, suggesting that maturation was not complete at this developmental stage. With the exception of DH receptors, there were significant differences between the stimulus-response functions of all the myelinated receptor types.
are incomplete and that considerable maturation of this type of receptor continues even from 2 wk after birth.

Response of thin myelinated and unmyelinated mechanosensitive afferent fibers to thermal stimuli

A subpopulation of unmyelinated and thin myelinated fibers was tested for their response to noxious cold or heat stimuli. Because the search procedure identified only the receptive field of mechanosensitive units (see METHODS), pure thermoreceptors were not included in the present analysis. However, we have noted that some fibers, which were probably unmyelinated as judged by the configuration of their action potential, that displayed a low level of irregular ongoing activity were vigorously excited by injection of cold fluid into the organ bath, resulting in small changes in the temperature. It is likely that these units are the sensitive cold receptors that constitute 5–10% of unmyelinated fibers in the rat; the prevalence of classical warm receptors is even lower in rodents and probably <1% (Kress et al. 1992). Thus unmyelinated units responding to both mechanical and thermal stimuli are probably best classified as polymodal nociceptors (Perl 1992).

Of 19 AM fibers tested, 5 (26%) were also activated by noxious heat (AMH units) and 1 of 10 units tested responded to noxious cold. There was no significant difference in the von Frey hair threshold between AMH (4.0 mN, interquartile range 2.8) or AM (4.8 mN, interquartile range 4.0) fibers, but AMH units had an average conduction velocity of
2.3 ± 0.2 m/s and thus tended to be at the lower end of the spectrum of Aβ-fibers.

The sensitivity to nonmechanical stimuli tended to be higher among unmyelinated than myelinated nociceptors. Of 17 C fibers tested, 7 (41%) were excited by the heat stimulus, and 3 of 10 C fibers responded to a cold stimulus. One unit responded to both thermal stimuli. There was no significant difference between the median von Frey hair threshold or the conduction velocities between the different subgroups of the C fibers.

Although both thin myelinated and unmyelinated fibers could signal the intensity of noxious heat, C units responded more vigorously. In both A and C fiber populations, the mean threshold of the units was the same regardless of whether it was defined as the temperature that evoked the second spike of the response or as the temperature that elicited an instantaneous frequency >1.0 imp/s. C units had a threshold of 37.6 ± 1.4°C, which was significantly lower (P < 0.05, t-test) than the 42.5 ± 1.9°C that was observed for A fibers. Moreover, the mean number of action potentials evoked during the standard heat stimulus in those fibers that were heat sensitive was 22.0 ± 6.0 for C units and 15.8 ± 9.7 for A units. Together with the fact that fewer A fibers responded to heat, the average stimulus-response function as calculated for the entire population of nociceptive A fibers was shifted to the right of that for C fibers (Fig. 9).

**DISCUSSION**

This is the first detailed investigation of the receptive properties of unmyelinated (C fibers) and myelinated (A fibers) afferent fibers in the mouse. A fibers were divided into four main groups of afferent fibers, namely RA and SA mechanoreceptors in the Aβ- and DH receptors and AM fibers in the Aδ-group. The proportions of A fibers classified into these four categories were remarkably constant in two cutaneous nerves of the hindlimb and in the two strains of mice.

**Receptor types among Aβ-fibers**

Aβ-fibers units had either RA or SA properties to a constant force stimulus. Previous studies in the cat have subdivided RA Aβ-fibers in the hairy skin into three major subdivisions, namely, hair follicle receptors, field receptors, and Pacinian corpuscles (Brown and Iggo 1967; Burgess et al. 1968; Perl and Burgess 1973; Tuckett et al. 1978). The last type was not encountered in this preparation, because the corpuscles in rodents and cats are most often located in deep subcutaneous tissues of the toes and feet (Burgess et al. 1968, 1974; Lewin and McMahon 1991), which are not preserved in this in vitro preparation. The subdivisions between field receptors (not activated by movements of hairs) and hair follicle receptors (which respond to hair movements) (Light and Perl 1993; Perl and Burgess 1973) was not possible, because individual hair follicles were not accessible for selective stimulation because of the inside-up mounting of the skin. However, the response properties of the large majority of RA units were similar to those of G hair units described in cat (Brown and Iggo 1967; Burgess et al. 1968) or rat (Baranowski and Lynn 1985; Leem et al. 1993; Lewin and McMahon 1991; Lynn and Carpenter 1982), which respond to movements of guard hairs. It is known that in the cat and the rat, field receptors are less sensitive than hair follicle receptors (Burgess et al. 1968; Lynn and Carpenter 1982; Tuckett et al. 1978), and in our experiments these may be represented as RA fibers with von Frey thresholds >1 mN (Fig. 5). However, because rat field receptors have mainly been found at the border between hairy and glabrous skin (Leem et al. 1993; Lewin and McMahon 1991) and because this tissue falls into the line of the cut in this preparation, it is unlikely that field receptors constitute a large fraction of the sample of RA fibers recorded in this study. Morphologically, the RA units may represent the palisade (or lanceolate) endings on hairs (Light and Perl 1993; Munger and Ide 1988). No attempt was made to distinguish between subcategories of G hair units (Burgess et al. 1968; Tuckett et al. 1978).

The SA Aβ-fibers found in the present study had no ongoing activity, discharged in an irregular pattern to a sustained stimulus, and responded to indentation rather than stretch of the skin. These fibers resembled in many aspects the type I SA unit found in the hairy skin of cat (Brown and Iggo 1967; Burgess et al. 1968; Iggo and Muir 1969) or rat (Baumann et al. 1993).
that have indicated that few nociceptive fibers have conduc-
tivity and this is in agreement with findings in other species
a week, when peripheral myelination is very advanced (Friede
et al. 1981), including humans (Adriaensen et al. 1983; Vallbo et al. 1995), DH receptors and nociceptors are the only mechano-
sensitive afferent fibers that found that have conduction veloc-
ities in the Aβ-range. Both DH and AM units are also present
in mice. A small minority of AM fibers did conduct at > 10
m/s, and this is in agreement with findings in other species
that have indicated that few nociceptive fibers have conduc-
tivity in the Aβ-fiber range (sometimes called Aβ-
nociceptors) (Koerber and Mendell 1992; Meyer et al. 1994;
Perl and Burgess 1973). These more quickly conducting AM
units only not have a different stimulus-response function
than SA units, they also have a broad action potential that
is characteristic of nociceptors (Koerber and Mendell 1992; Ritter and Mendell 1992). As in other species, a frac-
tion of the nociceptive AM units also responded to noxious
thermal stimuli. Whereas the receptive terminals of the AM
units are nonspecialized nerve endings at the dermal-epider-
mal border (Kruger et al. 1981), little is known about the
morbidity of the ends of DHs except that they are likely to
innervate hair follicles (Light and Perl 1993; Munger and
Ide 1988).

Receptor types of unmyelinated (C) afferent fibers

C fibers can be globally divided into thermoreceptors
(which lack mechanosensitivity) or mechanosensitive units
(Meyer et al. 1994; Perl and Burgess 1973). Although sensi-
tive cold receptors appeared to be occasionally present in
the recordings, all of the C fibers studied here in detail were
mechanosensitive. As in rat (Fleischer et al. 1983; Kress et al.
1992; Lewin and Mendell 1994; Lynn and Carpenter 1982),
cat (Beck et al. 1974; Bessou and Perl 1969), and primates
(Kumazawa and Perl 1977; LaMotte et al. 1982; Meyer and
Campbell 1981), including humans (Koltzenburg and Handwerker 1994; Schmidt et al. 1995; Torebjörk 1974), the C units of the mouse were further divided into subclasses on the basis of their response to noxious thermal stimuli. C fibers responding with high mechanical thresholds that respond also to nonmechanical stimuli are also known as polymodal nociceptors (Meyer et al. 1994; Perl and Burgess 1973). It is unresolved whether the few C fibers with me-
chanical thresholds of < 1 mN subserve a different (nonnoc-
ceptive) function (Bessou and Perl 1969; Kress et al. 1992;
Lynn and Carpenter 1982). Nociceptive afferent fibers in
the mouse had essentially the same characteristics as in other
species, and the distribution of von Frey hair thresholds and
thermosensitivity was very similar to that in rats. Interest-
ingly, we found here that quantitatively the responses of
AM and C fiber afferent fibers to suprathreshold mechanical
stimuli were essentially identical (Fig. 8), which is different
than the situation in cats (Garell et al. 1996) and primates
(Koltzenburg and Handwerker 1994; Slugg et al. 1994), where A fibers usually discharge more vigorously to me-
chanical stimuli. One possible explanation for this difference
in the present investigation may be that the stimulus was
applied from the corium side of the skin. However, in the
mouse, the average heat response of the C fiber population
was greater than for the A fibers, a situation which has also
been observed in rats (Lynn and Shakhabeh 1988).

Changes during postnatal development

In this study we also provide evidence that the receptive
properties of myelinated afferent fibers are not fully mature
in mice between 14 and 16 days old. Fitzgerald (1987)
showed in the rat that at the end of the second postnatal
week, when peripheral myelination is very advanced (Friede
and Samorajski 1968), the receptive properties appear ma-
ture. In agreement with these results, we found that the four
major types of A fibers could readily be distinguished at this
age in mice. As in the cat (Ferrington and Rowe 1980;
Ferrington et al. 1984), the quantitative characteristics of
these receptors as determined with quantitative mechanical
stimulation were similar, although not identical, to those in
afferent fibers in the adult. Except for the DH receptors,
there was evidence that the firing frequency to equivalent
stimuli was less in younger animals. This indicates that sub-
stantial maturation occurs over several postnatal weeks. For
SA fibers this was especially prominent, because they exhib-
ited a smaller and delayed peak response to increasing con-
stant force stimuli and also an elevated von Frey hair thresh-
old. These developmental changes occur at a critical period
in development when SA fibers require neurotrophin 3 for
survival (Airaksinen et al. 1996).

The most striking difference between the young postnatal
and adult afferent fibers was the presence of a receptor type
not encountered in adult animals, which we have called
RA/SA receptors. These afferent fibers behaved like normal
RA afferent fibers at low stimulus strengths (5–20 mN),
but become clearly SA at higher stimulus strengths (40–
200 mN). This unusual behavior was observed in almost
one-third of the more quickly conducting C fibers in the
young mice, and our evidence strongly suggests that these
receptors become conventional RA afferent fibers in adult
animals. These functional changes occur during a time of
substantial remodeling and maturation of the innervation of
hair follicles (Cassery et al. 1994; Payne et al. 1991). The
RA/SA fibers recorded in juvenile mice resemble, in some
aspects, RA fibers with abnormal adaptation properties found
in neuropathic conditions (Na et al. 1993). Postnatal
changes in the adaptation properties have also been shown
for muscle afferent fibers in rat pups (Vejsada et al. 1985)
and kittens (Jami et al. 1989; Skoglund 1960). However,
the transition from an SA to an RA pattern in a subpopulation
of cutaneous afferent fibers stands in marked contrast to the response properties of muscle spindle afferent fibers, which are initially very dynamic and become static as the receptors mature. It is possible that changes in afferent adaptation properties might be due to a continuous maturation process under the control of exogenous secreted factors and that the availability of neurotrophins, notably nerve growth factor (Koltzenburg et al. 1996; Lewin et al. 1992; Ritter et al. 1991), brain-derived neurotrophic factor (Koltzenburg et al. 1995), neurotrophin 3 (Airaksinen et al. 1996), and neurotrophin 4 (Stucky et al. 1996), or their receptors (Koltzenburg and Stucky 1996), is instrumental for this maturation.

In summary, we have presented the first comprehensive description of the properties of mouse cutaneous receptors. The receptive properties of these afferent fibers appear to be very similar to those described in rats. Although the receptive properties can readily be distinguished at the end of the second postnatal week, the afferent fibers are not fully mature and there are still substantial changes in adaptation properties and discharge frequencies. The changes we have observed in the receptor properties between 2-wk and adult animals suggest that continuing adjustments might have to be made postnatally between the peripheral nervous system and the CNS in growing animals (Lewin and Mendell 1996). This preparation will enable further detailed studies in transgenic mice to determine the consequences of eliminating or adding specific gene products on the maturation of afferent function.

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REFERENCES


