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Merkel Cell Receptors: Structure and Transducer Function

Abstract. *An electron microscopic and electrophysiological investigation was made of Merkel cell–neurite complexes in the sinus hair follicles of the cat. These mechanoreceptors respond with very precise phase locking to high-frequency vibratory stimuli as well as to static hair displacements. The mechanoelectric transduction process is faster than that known for any other somatic mechanoreceptor. These data show that the nerve endings themselves and not the Merkel cells are the mechanoelectric transducer elements in these receptors.*

The structure and function of Merkel cells (MC's), which occur in association with a distinct type of mechanoreceptor in vertebrate skin, have been debated ever since Merkel first described the cells in 1875 (*1*). In mammals MC's are most abundant in the epidermal touch corpuscles and in the external root sheath of sinus hairs (vibrissae) (*2–4*). In both places MC's are contacted by nerve endings to form slowly adapting mechanoreceptors. These complexes have similar response properties in all mammalian species so far investigated (*5*). However, it is not known whether MC's are the mechanosensitive transducer elements of the receptor (*6*). On the basis of ultrastructural evidence, it was suggested that MC's could be secondary sensory cells (*3, 4*). Since the cytoplasm of MC's is rich in dense-core granules, it was assumed that mechanical deformation of the MC's would cause the generation of a receptor potential, which would be transmitted to the nerve endings through the release of a chemical transmitter from the dense-core granules at specialized zones of contact between the MC's and the nerve endings. However, since the nerve endings morphologically resemble those in other types of mechanoreceptors, a second possibility is that the mechanoelectric transduction process may take place at the nerve endings and not at the MC's (*3*).

Electrophysiological investigations have supported both these hypotheses. In newborn kittens afferent responses were recorded from touch corpuscles before the MC's had differentiated (*7*), and it was concluded that the nerve endings were the mechanoelectric transducer elements. In contrast, Horch *et al.* (*8*), who studied the discharge and excitability characteristics of afferent responses elicited from touch corpuscles, concluded that the action potentials were generated through a synaptic mechanism. They considered the MC's to be the receptor elements, and this interpretation is now generally accepted, even though it conflicts with several other findings. For instance, MC's survive denervation unaltered for several months (*9*), which is unusual for secondary sensory cells (*10*). Identification of a transmitter substance in the dense-core granules has long been unsuccessful, and the supposed synaptic transmission has not been blocked (*4, 6, 11*). Recently, however, a "Met-enkephalin–like" substance was demonstrated histochemically in MC's (*12*), and this finding, together with an ultrastructural verification of "synapses" between MC's and nerve endings, suggested anew that MC's function as receptor cells (*13*). We obtained new evidence against this concept by performing an electron microscopic and electrophysiological reinvestigation of the mechanoreceptors in the sinus hair follicle of the cat.

A cat was perfused with 2.5 percent phosphate-buffered glutaraldehyde and several sinus hair follicles were removed, embedded in Araldite, and cut transversally or longitudinally in serial semithin or ultrathin sections. Examination with an electron microscope confirmed previous observations concerning the ultrastructure of MC-neurite complexes (*2, 3, 14*), but in our view the morphological details suggest that their function is quite different from that of sensory cells. The MC's are situated in a regular manner in the external root sheath and are far more numerous—about 3000 were counted even in small sinus hair follicles—than has been suggested. Only 50 to 70 percent of them contact a nerve terminal, which then always lies on that side of the MC which is directed toward the follicle's orifice. If the MC's have a receptor function, not so many would lack a nerve contact.

The distribution of dense-core granules in the cytoplasm of MC's is always alike, whether a nerve contact exists or not. The dense-core granules are most numerous in that part of the MC's which might also be contacted by the nerve endings; usually they are concentrated in the peripheral cytoplasm and often outside the zone of contact with the nerve terminal (Fig. 1A). Thus the distribution of dense-core granules in the MC's appears to be independent of the presence of a nerve ending and inappropriate for the disposition and release of a transmitter substance. Such release is supposed to take place at "synapse-like" membrane contacts between MC's and nerve endings (*3, 4, 6, 13*). These specialized zones are situated mostly at the periphery of the disklike nerve terminals, but can be found with (Fig. 1B) and without (Fig. 1C) dense-core granules at the presumptive presynaptic side. Thus a specific affinity between the zones of contact and dense-core granules is not apparent. Also, for the operation of a synaptic mechanism in a receptor that can sustain a 1200-Hz discharge, many more dense-core granules would have to accumulate at the supposed transmitter release sites than are generally visible.

The synapse-like structures may, in fact, be desmosome-like attachment points between MC's and nerve endings. [The historical interpretation is understandable in view of the difficulty in distinguishing between true synaptic and merely adhesive cell contacts (*15*).] The MC's thus may function not as sensory cells but as abutments for the deforma-

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tions of the mechanosensitive nerve endings. This possibility is morphologically supported by the connection of MC's with the tonofilament system of epidermal keratinocytes—mediated by numerous desmosomes (Fig. 1D)—and by the microfilamentous cytoskeleton in the MC's, which extends into the many microvilli protruding from the MC surface. Thus the MC's should possess special viscoelastic properties and the mechanoelectric transduction function should be allocated to the nerve endings. Indeed, mitochondria are abundant in the nerve endings (Fig. 1A), and a finely granular axoplasmic structure, the so-called receptor matrix (Fig. 1, A and D), is present. Both of these characteristics are typical of sensory nerve endings in other vertebrate mechanoreceptors (*16*).

We obtained evidence that the mechanoelectric transduction process occurs at the nerve endings by recording responses of different kinds of mechanoreceptors in the sinus hair follicle from single axons isolated from the infraorbital nerve (*17*). Single vibrissae were moved by trapezoidal or sinusoidal stimuli from a feedback-controlled electromechanical stimulator. The vibratory stimuli had frequencies between < 1 and 3500 Hz and maximal amplitudes of 220 μm at 500 Hz and 50, 12, and 5 μm at 1, 2, and 3 kHz, respectively.

When a sinus hair is bent in opposite directions and then held in position, the type 1 nerve fibers, which terminate on MC's (*17*), give rise to a directionally sensitive, slowly adapting response (Fig. 2A). Vibratory stimuli elicit phase-coupled responses, which occur at stimulating frequencies below 80 Hz as multiple discharges per vibration cycle (Fig. 2B) and above 80 Hz as one impulse per sine wave. Such 1:1 responses were elicited readily at 1200 Hz (Fig. 2B) for stimulating periods of 500 msec. Responses to vibratory stimuli with even higher frequencies (up to 2800 Hz) were not uncommon, but above 1500 Hz the afferent nerve fiber could not maintain a 1:1 discharge due to its inability to conduct impulses within the refractory period (*18*).

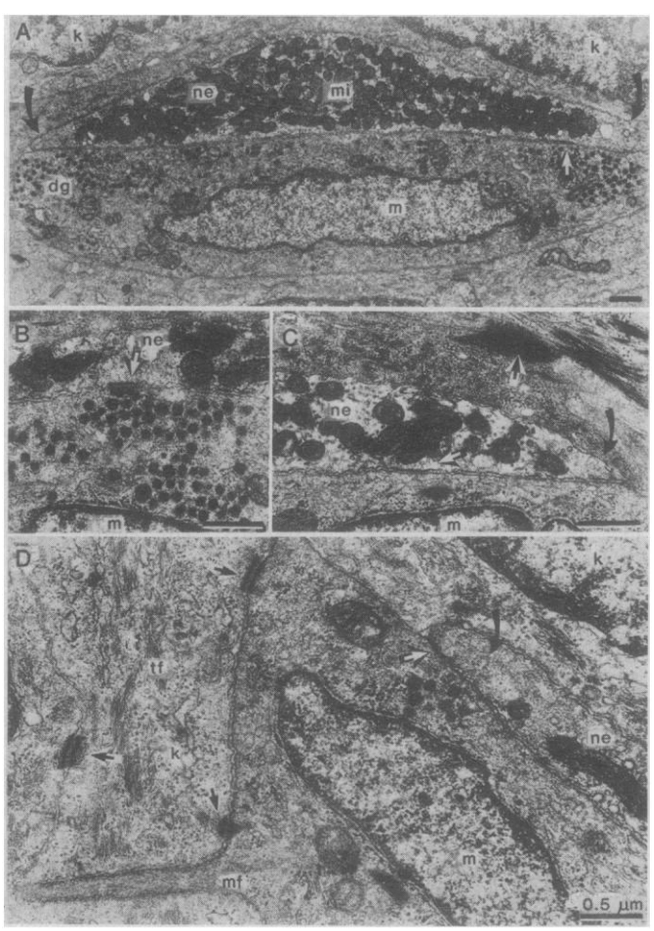

Fig. 1. Electron micrographs of MC-neurite complexes in the sinus hair follicle of the cat. (A) The MC's (*m*) contain many dense-core granules (*dg*), while the nerve endings (*ne*) are densely packed with mitochondria (*mi*) which are missing at the lateral edges of the nerve endings (curved arrows), where instead a typical receptor matrix is observed (*16*). Specialized membrane contacts occur between the MC's and the nerve endings (white arrows), with (B) or without (C) accumulated dense-core granules at the MC side. (D) The MC's are surrounded by keratinocytes (*k*), which contain bundles of tonofilaments (*tf*) converging on desmosomes (black arrows) that connect the keratinocytes with each other or with the MC's. The latter possess straight, microvilli-like processes containing microfilaments (*mf*), which are continuous with a microfilamentous cytoskeleton.

The phase locking with which consecutive impulses were elicited in high-frequency responses was remarkably precise. This is illustrated in Fig. 2C, which shows superimposed impulses occurring at the same cycle of at least ten repeated vibratory stimuli. The precision of phase locking of the individual impulses in a vibratory response increased gradually with the frequency, and in the 1:1 response to a 900-Hz stimulus the phase jitter was less than 10 μsec. Such low phase jitter was also preserved in responses to vibratory stimuli above 1500 Hz, indicating that the receptor mechanism operating in MC-neurite complexes is able to resolve and follow vibratory frequencies far above 1500 Hz.

The characteristics of the vibratory responses can hardly be explained in terms of chemosynaptic transmission (*19*). It is unlikely that a transmitter release mechanism could operate in phase with a vibratory stimulus of 1200 Hz or more. Also, at such frequencies an accumulation of released transmitter would be likely. This would cause a temporal summation of postsynaptic responses, resulting in a phase jitter of the afferent impulses which increased rather than decreased with the frequency of the vibratory stimuli. The argument that transmitter accumulation would not occur because of very fast removal conflicts with the fact that the same receptor can sustain a tonic discharge of low frequency in response to constant bending of a sinus hair. Moreover, the electron microscopic findings cannot be considered to show a convincing morphological substrate for the observed high-frequency vibratory responses.

It was also possible to measure the duration of the mechanoelectric transduction process for the different receptor types in the sinus hair follicle. The rationale of such measurements has been to see whether, in any type of mechanoreceptor—and especially in the MC-neurite complexes—chemosynaptic transmission can take place in the measured transduction times.

To determine the receptor delay (*20*)—in this case the difference between the response latencies following mechanical stimulation of the hair shaft and electrical stimulation of the afferent nerve fiber—we inserted a bipolar steel electrode into the skin on either side of the sinus hair follicle. A brief mechanical stimulus consisting of only one movement cycle of a high-frequency vibration (1000 to 2500 Hz) was applied to measure the mechanical response latency (Fig. 3, A, C, and E). To ascertain that a response was effectively elicited at the

beginning of the mechanical stimulus, two opposite directions of initial movement were used. The mechanical response latency was the time between the onset of the displayed electronic stimulus signal and the shortest response.

An example of the results of such measurements for a type 1 nerve fiber is given in Fig. 3A. The typical directional sensitivity of this MC-receptor afferent is reflected in two different response latencies, since only the downward-going wave component moved in the sensitive direction. As indicated by the shaded area, the receptor delay in this example is 0.3 msec. The shortest receptor delay observed in a type 1 nerve fiber was 0.2 msec. If we assume a mechanical delay of about 0.1 msec between onset of the electronic signal initiating movement of the stimulator and onset of the receptor deformation, the delay caused by the mechanoelectric transduction process amounts to 0.2 msec in the type 1 unit illustrated in Fig. 3A.

We also show the results of similar measurements for a slowly adapting type 2 fiber (Fig. 3C) and a rapidly adapting fiber (Fig. 3E), both of which innervate sinus hair follicles but derive from mechanoreceptors other than MC-neurite complexes (*17*). These two receptor types are not directionally sensitive. The somewhat longer delay times are presumably due to larger mechanical delays, resulting, for example, from greater distances between the intrafollicular hair end and the receptors within the inner hair follicle. However, in all three receptor types a transduction process having a similar time course seems to be operative. In addition, all three receptor types can respond to vibratory stimuli of 1000 Hz with a 1:1 discharge and a comparably low phase jitter (Fig. 3, B, D, and F), suggesting that in the different kinds of mechanoreceptors the same basic transduction process takes place.

The receptor delay measurements indicate that no chemosynaptic transmission of a receptor potential precedes the generation of afferent impulses in MC-neurite complexes. A receptor delay of 0.2 or even 0.3 msec appears to be too short for a chemosynaptic transmission to occur (*19*). Theoretically, the available time would permit electrical coupling between MC's and nerve endings, but there is no morphological evidence for such coupling. The electrophysiological findings show that the transduction process can only occur directly at the nerve endings. This has already been suggested for other types of mechanoreceptors (*21*). In addition, the receptor delay has proved far shorter than would be expected from measurements of mechanoreceptors occurring in locations other than the sinus hair follicle (*20*).

The functional significance of MC's is still unclear. Undoubtedly they have an affinity for nerve endings (or vice versa), perhaps because they subserve a trophic or other supportive function. This is suggested by our recent finding that in sinus

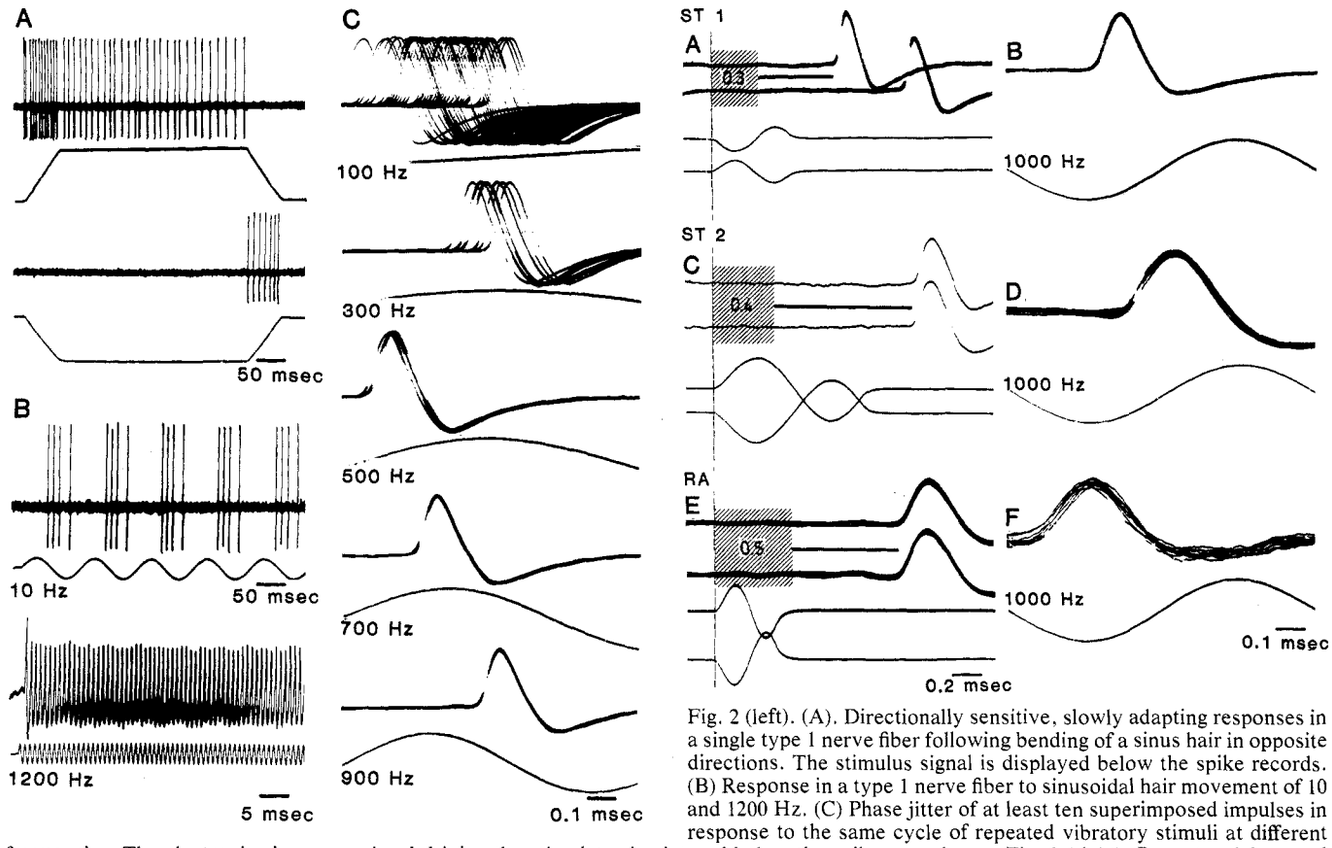

Fig. 2 (left). (A). Directionally sensitive, slowly adapting responses in a single type 1 nerve fiber following bending of a sinus hair in opposite directions. The stimulus signal is displayed below the spike records. (B) Response in a type 1 nerve fiber to sinusoidal hair movement of 10 and 1200 Hz. (C) Phase jitter of at least ten superimposed impulses in response to the same cycle of repeated vibratory stimuli at different frequencies. The electronic sine wave signal driving the stimulator is pictured below the spike records. Fig. 3 (right). Receptor delays and phase locking in high-frequency responses of three receptor types in the sinus hair follicle of the cat. Parts (A), (C), and (E) show responses following two single sine wave stimuli of high frequency moving a sinus hair in opposite directions. The upper spike records relate to the upper stimulus signal, the lower records to the lower stimulus signal. Single traces are shown in (A) and (C), and ten superimposed traces are shown in (E). The response latency of the afferent nerve fiber following an electrical stimulus close to the sinus hair follicle is indicated by the black bar between the spike records. The width of the hatched area, given in milliseconds, indicates the duration of the receptor delay. The response latency in the lower record of (A) is somewhat longer than expected, because with just one movement cycle the stimulator, although actuated almost instantaneously with the sine wave signal, could not follow its high frequency (2000 Hz). (B, D, and F) One-to-one discharges with low phase jitter in the three receptor types following a 1000-Hz vibratory stimulus. Each record represents ten superimposed impulses occurring at the same cycle of the repeated vibratory stimuli; *ST1* and *ST2*, slowly adapting sinus hair type 1 and type 2 fiber, respectively; *RA*, rapidly adapting sinus hair follicle fiber.

hair follicles of the newborn kitten there are 60 percent fewer MC's and nerve endings than in the adult, while the number of myelinated axons entering the follicle is about equal in young and adult animals. Therefore, during maturation new MC's must be formed together with new branches of the afferent nerve fiber. Presumably, MC's play a role in this postnatal arborization of the type 1 nerve fiber, perhaps by serving as targets for new nerve endings (*22*) and then maintaining the resulting branching pattern. For such function a substance stored in the dense-core granules might be released from MC's, but such a release would not be part of the mechanoreceptive transduction process. A role of MC's as passive abutments for the nerve endings concurs best with the electrophysiological and morphological observations.

In more general terms, our results show that the type 1 and type 2 receptors in the sinus hair follicle respond not only to a wide range of amplitudes for dynamic and static hair displacements (*17*) but also to a wide range of frequencies (from nearly 0 to about 1500 Hz). Thus, in addition to their function as displacement detectors, they respond to a range of vibratory stimuli hitherto thought to be detected only by Pacini-type cutaneous mechanoreceptors (*23*).

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Rheumatoid Factor–Like Immunoglobulin M Protects Previously Uninfected Rat Pups and Dams from *Trypanosoma lewisi*

Abstract. *The serum of lactating rats that have never been infected with the protozoan parasite* Trypanosoma lewisi *contains a rheumatoid factor–like immunoglobulin M (IgM). This IgM amplifies a specific immunoglobulin G (IgG) response to the parasite and accounts for the unusual resistance of previously uninfected lactating rats and their suckling pups to infection with* T. lewisi. *A similar rheumatoid factor–like IgM, which is induced late in the usual course of infection with* T. lewisi *in nonlactating rats, amplifies an earlier IgG response and terminates the infection. To our knowledge, this is the first description of a rheumatoid factor, which is classified as an autoimmune antibody, acting in a protective manner.*

We showed previously (*1*) that lactating rats have an unusual resistance to *Trypanosoma lewisi*, a flagellate protozoan parasite that is highly specific for rats. In particular, we found that lactating rats have a lower peak parasitemia and do not develop the second or "adult" phase of the parasitemia; thus infections in these rats last for less than half the usual time, that is, 12 days as opposed to 24 to 26 days. We also found that the serum of lactating rats that have never been infected with *T. lewisi* can agglutinate parasites of the adult phase isolated from infected, nonlactating rats. Furthermore, we observed that suckling rats infected with *T. lewisi* have a 100 percent survival rate if they are infected at 10 days of age (about the midpoint of the suckling period), but only a 76 or 55 percent survival rate if they are infected at 15 or 17 days, respectively (toward the

Table 1. Identification of the lactating rat serum factor as an IgM. Titers are expressed as reciprocals of the maximum dilution of serum or serum fraction that produced clumps of four or more parasites in every microscope field examined. Serum samples from nonlactating rats were always negative.

Treatment	Titer before treatment	Titer after treatment
Exposure to 56°C for 30 minutes	32	32
Exposure to 65°C for 30 minutes	32	0
Precipitation by 40 percent saturated $(NH_4)_2SO_4$ and redissolution of precipitate to the original volume	64	32
Three adsorptions with 5×10^8 adult parasites per adsorption per milliliter of serum	64	0
Incubation in 0.2*M* mercaptoethanol followed by dialysis against 0.02*M* iodoacetate in 0.85 percent NaCl	32	0
G-200 Sephadex chromatography	Activity recovered only in first peak, indicating a molecular weight of > 600,000	
Prior incubation of lactating rat serum (0.2 ml) with goat antiserum to rat IgM (0.1 ml)	64	0
Control incubation with normal goat serum	64	64

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