ANIMAL PAIN
Perception and Alleviation

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Preface

Pain is a complex physiological phenomenon; it is hard to define satisfactorily in human beings, and it is extremely difficult to recognize and interpret in animals. Scientific knowledge concerning pain perception in animals must be obtained by drawing analogies based on comparative anatomy, physiology, and pathology and by inference based on subjective responses to pain experienced by humans. Debate continues about whether animals of different species perceive pain similarly and whether any species perceives pain the same way humans do. The use of animals in research, in education, and in testing products to minimize adverse effects requires more knowledge about pain perception in animals. Increasing public concern about animal welfare has added urgency to this need.

Our knowledge of the scientific basis of the mechanisms of pain has advanced substantially in the last two decades. Nociceptors, or pain receptors, are widespread in the skin and tissues of animals; chemical mediation of nociceptor excitation may provide a key to understanding the peripheral phenomena related to pain. The expression of pain in animals involves multiple ascending and descending branches as well as specialized pain-signaling mechanisms in the spinal cord. The importance of these different pathways varies with species and circumstances. Endogenous neural systems in the brain stem and forebrain, including both opioid and nonopioid mechanisms, may modulate the central transmission of nociceptive signals in animals. Noxious stimuli mediate a variety of different functions; each animal has a consistent response to noxious stimuli or a consistent pattern of escape from pain.

A symposium on pain in animals was held at the 66th Annual Meeting of the Federation of the American Societies for Experimental Biology (FASEB) in New Orleans on April 20–21, 1982. Other workshops and meetings on the subject of pain have focused primarily on pain in humans; this is the first symposium known to us that has specifically addressed pain in animals. The American Veterinary Medical Association (AVMA) Council on Research, with financial support from the AVMA Foundation, planned and organized the symposium.

Cosponsors were the American Physiological Society and the American Society for Pharmacology and Experimental Therapeutics, constituent members of FASEB, who contributed suggestions on planning and organization.

The goal of the symposium was to establish the factual background on acute pain in animals from which more detailed and specific information can be developed. Chronic pain in animals was not specifically addressed, but the symposium did project research needs to better measure and alleviate pain, discomfort, and anxiety in experimental animals. As the mechanisms of pain are better understood, the humane treatment and alleviation of pain in experimental animals can be placed on a much firmer scientific basis.

The book is divided into two sections. Pain perception in animals focuses on peripheral and supraspinal mechanisms involved in pain, segmental neurophysiological mechanisms, spinal cord pathways and control systems, stimulation analgesia and endorphins, behavioral mechanisms for assessment of pain, assessment of pain during surgical procedures, and phylogenetic evolution of pain expression in animals.

Alleviation of pain in animals covers drug-disposition factors and species variation, evaluation of analgesic drugs in horses, and control of pain in dogs and cats. There is a significant variation among species in the absorption and biotransformation of drugs used to alleviate pain in animals.

This book is intended as a source of basic information about the perception and alleviation of pain in animals for scientific investigators working in this area; for veterinarians interested in the health and welfare of animals, the assessment of pain during surgery, and the alleviation of pain; and for individuals involved in federal, state, and local regulation of the use of animals in research, education, and quality control of human and animal health products.

As chairman of the planning committee for the symposium, I thank the session chairmen, speakers, members of the planning committee (L. E. McDonald and P. K. Hildebrandt), other members of the AVMA Council on Research, R. L. Kitchell (consultant to the Council), and E. R. Ames (AVMA staff consultant).

We are grateful to the AVMA Foundation for financial support of the symposium. We thank the American Physiological Society, O. E. Reynolds, S. R. Geiger, editors, and staff for assistance in meeting arrangements and the publication of this book.

Howard H. Erickson

Introduction: What is Pain?

Pain in animals is a topic of considerable interest and debate involving strong human emotions affected by differing personal beliefs of individuals with widely divergent backgrounds. Any discussion of pain involves semantics. Pain has been defined by different individuals based on personal experiences. Most authorities agree that pain is a perception, not a physical entity, and that perception of pain depends on a functioning cerebral cortex. Unlike most other sensations, no single area of the cerebral cortex seems specifically necessary for the perception of pain. It is semantically incorrect to refer to "pain" stimuli, impulses, pathways, or reflexes because these occur in humans, and presumably in animals, without perception of pain. The term *noxious* describes stimuli that, if perceived, give rise to the perception of pain. Sherrington (3) originally defined noxious as a stimulus actually or potentially damaging to the skin. The receptors specifically responsive to noxious stimuli are termed *nociceptors*. A stimulus must be a certain strength before a nociceptor will generate nerve impulses in the peripheral nerve fiber of which it is a part. This stimulation strength is called the *nociceptive threshold*. In certain circumstances this amount of neural activity may be too little to result in perception of pain. The strength at which noxious stimulation is perceived by a human being as pain is referred to as the *pain detection threshold*. The strongest intensity of noxious stimulation that a human being will permit an experimenter to deliver is called the *pain tolerance threshold*. The strength of noxious stimulation necessary to reach the nociceptor threshold is rather constant and varies little among humans and animals. The strength needed to cross the pain detection threshold is slightly more variable, especially among humans experiencing clinical pain (4). The pain tolerance threshold is the most variable of the three thresholds. Most clinical veterinary neurologists are amazed by the high pain tolerance thresholds of some dogs.

Introspective verbal reports are the most frequently used method of assessment of pain perception in human beings. Some authorities have developed objective measurements (e.g., manipulation of a lever or

drawing lines of varying length) to better quantitate pain detection and pain tolerance thresholds. When considering pain in animals, analogies must be drawn between human and animal anatomy, physiology, and behavior. Knowledge about pain in animals remains inferential, however, and neglect of the probabilistic nature of pain perception in animals leads to anthropomorphism (1). On the other hand, overemphasis on the uncertainty of our knowledge about pain perception in animals, which leads to a denial that pain perception exists in animals, is logically as well as empirically unfounded (1). The tacit assumption is that stimuli are noxious and strong enough to give rise to the perception of pain in animals if the stimuli are detected as pain by human beings, if they at least approach or exceed tissue-damaging proportions, and if they produce escape behavior in animals (2).

This book describes the neuromechanisms involved in pain perception and alleviation in animals. It is hoped that this volume will inspire additional studies and symposia dedicated to developing better methods for the assessment of pain and its prevention and alleviation.

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Peripheral Mechanisms Involved in Pain

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Specificity Versus Pattern Theory • Anatomy and Physiology of Nociceptors: Small myelinated afferents, Unmyelinated afferents, Chemical mediation • **Pathology Associated With Nerve Injury:** Peripheral sprouting of nociceptive afferents in noninjured nerves, Neuroma formation

The structural and physiological basis of pain sensations has been the most elusive area of sensory research, and the information concerning specific receptors and pathways is mostly recent. The nineteenth century concept of sensory modality, introduced by Helmholtz to designate qualitatively distinctive sensory continua, was widely adopted to fit a schema in which touch, warmth, cold, and pain were accepted as primary qualities or modalities (10, 68, 107) in keeping with the Müller concept of specific energy. Müller's concept is more easily applied to the organs of special sense than to cutaneous sensation because it is often difficult to excite the skin and evoke a single introspective sensory quality; for example, it is difficult to evoke pain without touch or warmth components. The discovery by Blix (8) of a mosaic of sensory-specific spots ultimately led to acceptance of the idea that there was a distinctive anatomical substrate subserving each specific modality. Von Frey allocated the end organs known in the nineteenth century to each of the established modalities (68, 98, 107).

Specificity Versus Pattern Theory

The von Frey theory has been continually challenged on numerous grounds. The separability of cold and warm spots seemed clear enough, but the density of touch and pain even led Blix to doubt their spatial

distinctiveness. The von Frey assignment of Krause end bulbs to cold sensations and Ruffini endings to warm sensations has been challenged by modern knowledge, but his assignment of free nerve endings to pain is still widely accepted, and his association of hair follicle basket formations and Meissner corpuscles to touch in hairy and glabrous skin, respectively, is almost certainly a correct, if incomplete, correlation. Goldscheider (39) challenged the problem of specificity with particular reference to pain, because pain could be evoked by a variety of stimuli if they were sufficiently intense. The concept of pain as an affective component distinct from a sensory modality has survived into the modern era, although it has long been recognized that there are conditions (e.g., syringomyelia, cocaine and nitrous oxide analgesia) in which an absence of pain is not accompanied by loss of other sensory qualities.

The introduction of electrophysiological techniques helped resolve some of the conflicts, and Adrian's pronouncement of the "all-or-none law" in peripheral nerve axons, followed by the pressure and anesthetic nerve-block experiments of Gasser and Erlanger, provided new support for the Sherringtonian doctrine that a specific adequate stimulus could be determined for each peripheral axon. In the 1950s several workers defined the adequate stimulus for axons with pain endings in terms of their clearly elevated thresholds to all stimuli (54, 55, 59, 81). However, many investigators uphold the view that each receptor responds to a range of stimuli, which it converts into patterns of axonal impulses rather than into specific-line modality information, and that the discharge pattern from several receptors is essential for sensory discrimination (82, 83).

In the following decade the laboratories of Iggo and Perl established the existence of coherent populations of visceral and cutaneous receptors with unmyelinated or thinly myelinated axons that possess elevated thresholds for innocuous stimuli and that discharge vigorously only in response to noxious stimulus intensities (16). The existence of nociceptive cutaneous sense organs has been confirmed in the past decade for many species, including humans, and the features of myelinated and unmyelinated classes have been characterized in several laboratories (107). These data are consistent with human experiments demonstrating that electrical stimulation of slowly conducting myelinated fibers elicits fast, pricking pain; slow, burning pain is elicited in unmyelinated fibers when repetitively stimulated (7, 28). Although there is no report of unpleasantness or pain until the thinly myelinated fibers are excited (28, 42), stimulation of only the faster A fibers correlated with tactile sensation can produce both pain and nociceptive reflexes (127) when excited repetitively; a few fast-conducting

myelinated fibers appear to display high thresholds (51). Recent evidence relating individual fibers to specific sensations is derived from the observations of Torebjörk and Ochoa (116) in which microelectrode recording from single fibers in humans revealed that the natural adequate stimulus for eliciting discharge in each fiber correlated accurately with the sensation aroused by electrically stimulating the same fiber. A crucial observation is that excitement of only one myelinated or unmyelinated nociceptor axon is necessary to produce pain sensation without invoking a complex pattern of multiple inputs.

Anatomy and Physiology of Nociceptors

The prevailing nineteenth century view that the morphological substrate of pain can be assigned to free nerve endings still survives largely because of their ubiquitous distribution and especially because pain can be elicited from zones of hairy skin lacking the corpuscular receptors commonly found in glabrous skin (108). The terms *free*, *bare*, and *naked* were originally derived principally from studies with silver impregnation and, to a limited extent, vital staining with methylene blue. The deficiencies of both methods have been reviewed by numerous authors (41, 89, 107). It is generally recognized that metallic impregnation is often erratic and incomplete in delineating unmyelinated fibers and additionally is subject to artifacts of overimpregnation, including staining of collagen and reticular fibers. Methylene blue provides more specific axonal visualization but fails to reveal many of the endings that are apparent with acetylcholinesterase histochemistry (29). Our current investigations, involving the axonal transport of a horseradish peroxidase–lectin conjugate, suggest that both metallic impregnation and vital stains provide an incomplete picture. In any case, these light-microscopic methods do not even reveal the envelopment of axons by Schwann cells. Several authors have suggested that the term *free nerve ending* is misleading and should be discarded because it implies that the axon is bare, a condition that does not exist when these endings are examined with the electron microscope (20, 69, 94). It is now recognized that a complete description of a cutaneous nerve ending and its surround can only be achieved through electron-microscopic examination.

The limitation of morphological studies makes correlating structure and function difficult, but identification of physiologically characterized receptive-field spots has been achieved with electron-microscopic methods for the description of the Merkel cell complex of Pinkus-Iggo domes (57) and cold spots (47). A similar approach seemed applicable to one class of fibers implicated in pain (69).

Small myelinated afferents

Cutaneous mechanical nociceptors (also known as high-threshold mechanoreceptors), with afferent fibers conducting between 5 and 50 m/s, constitute a functionally congruent class in the hindlimb nerves that innervate the hairy skin of cats and monkeys (15, 97). Their average conduction velocity of under 30 m/s (Fig. 1) corresponds to the fastest component of the δ-wave of the compound action potential. This feature has led to calling these afferent units δ-nociceptors, although those with lowest threshold and highest conduction velocity may extend slightly beyond the δ-nociceptor range.

The receptive field of each myelinated nociceptor afferent unit consists of an array of spots, each causing a response when indented by a strong mechanical stimulus (Fig. 2). Each excitable point (usually <250 μm) is surrounded by an inexcitable region from which responses cannot be elicited with stimulus intensities several times greater than those sufficient to activate the responsive spots (Fig. 3). Mapping the discontinuous nature of the receptive field requires the use of small, sharp probes and avoidance of skin penetration and damage to prevent receptor inactivation. Repeated stimulation may also enhance their responsiveness (4, 18, 35), a process commonly referred to as sensitization.

A series of 17 such spots, mapped and bracketed by the insertion of stainless steel insect pins, was studied in the distribution of the posterior femoral cutaneous nerve of the cat hindlimb. A search was made for an isolated thinly myelinated axon in semithin plastic sec-

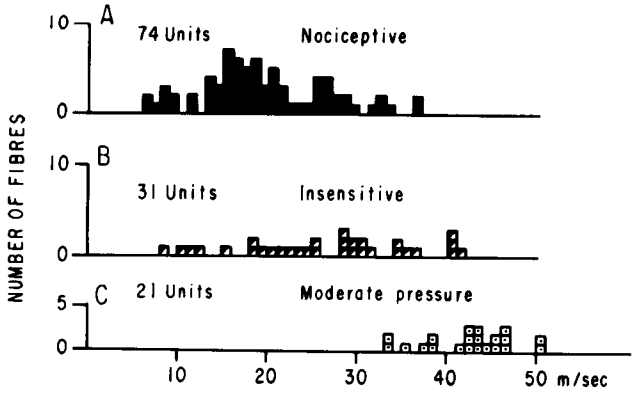

Fig. 1. Conduction velocities of high-threshold mechanoreceptor fibers in cat hindlimb. Those fibers with highest threshold in nociceptive range tend to cluster at low range of conduction velocity; those requiring moderate pressure tend to cluster at high range of δ-fibers and above. [From Burgess and Perl (15).]

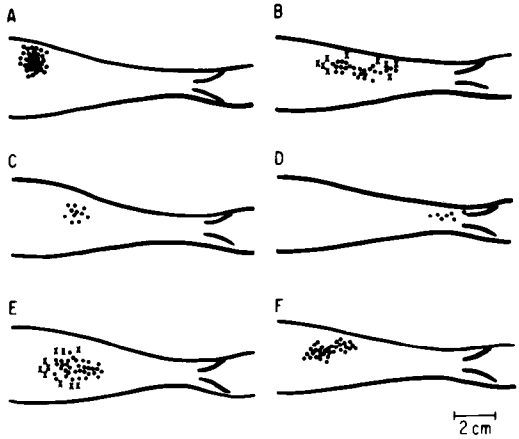

Fig. 2. Receptive fields of 6 thinly myelinated nociceptor fibers innervating cat hindlimb. Each receptive field consists of a variable cluster of spotlike zones surrounded by insensitive domains. Conduction velocities 9.5–29 m/s. [From Burgess and Perl (15).]

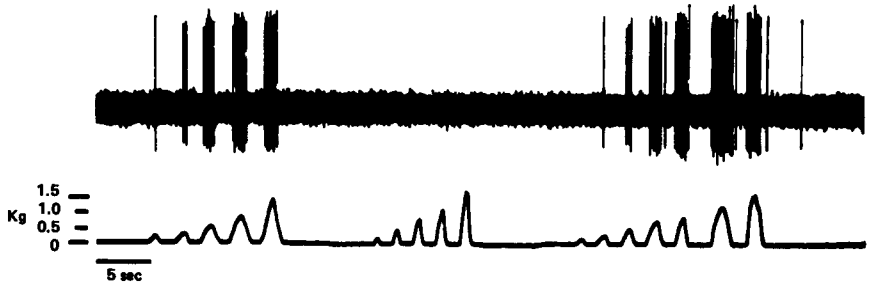

Fig. 3. Discharge from 2 adjacent spots (5 mm apart) of a 17-spot receptive field recorded from myelinated nociceptor fiber in cat (*upper trace*), demonstrating discontinuous nature of receptive field. Force applied with 1-mm probe (*lower trace*) to 1 spot (*upper left*) reveals unresponsive intermediate zone (*middle*) and similar response at nearby spot (*right*). Force values are reduced with use of sharp probe but with risk of receptor damage. [From Kruger, Perl, and Sedivec (69).]

tions of each block, with two pinholes used for orientation. Subsequent semithin and thin sections were traced by following an axon leaving a mixed bundle in its upward course into the dermal papillary layer within the marked zone (Fig. 4). The distal unmyelinated continuation of a thinly myelinated axon into one of the papillary ridges within the demarcated zone was consistently found in each marked spot. Although the dermal papillae within this zone contained other specialized structures, the most consistent feature of each block examined was the presence of several unmyelinated-axon profiles surrounded

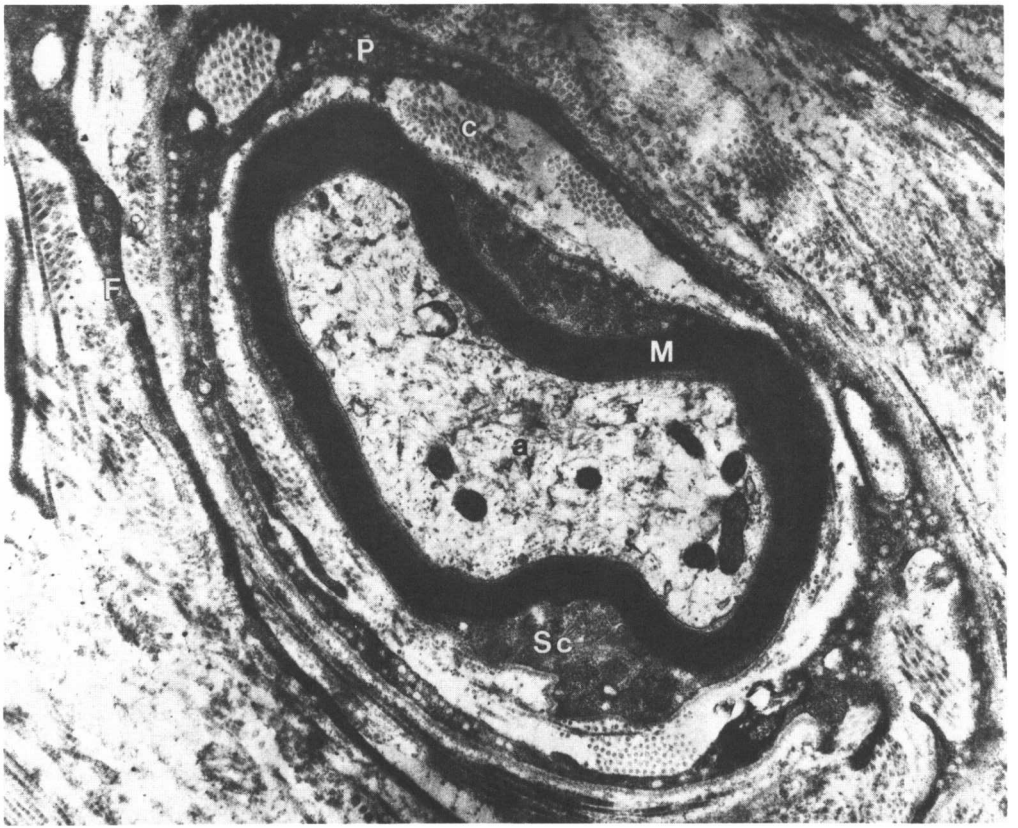

Fig. 4. Electron micrograph of isolated small myelinated (*M*) axon (*a*) and its Schwann cell (*Sc*) in subpapillary dermis below marked nociceptor spot, surrounded by perineurial sheath (*P*), fibroblasts (*F*), and endoneurial collagen (*c*). ×12,700. [From Kruger, Perl, and Sedivec (69).]

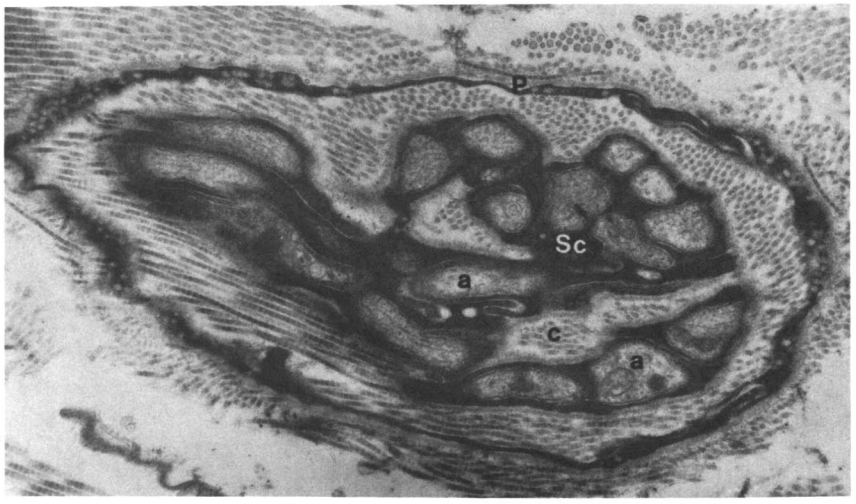

Fig. 5. Electron micrograph of typical dermal unmyelinated nerve bundle with numerous axons (*a*) surrounded by single Schwann cell (Sc), endoneurial collagen (*c*), and thin layer of perineurium (*P*). ×11,800. [From Kruger, Perl, and Sedivec (69).]

by a thin layer of Schwann cell cytoplasm and its basal lamina (Fig. 5). The several profiles may belong to a single undulating fiber, its branches, or a bundle of separate axons, but their failure to disperse widely suggests that this arrangement is a feature characteristic of myelinated mechanical nociceptors.

The axon–Schwann cell complex was traced to the epidermal border where the thin Schwann cell basal lamina merges with the thicker, denser basal lamina underlying the basal keratinocytes (Fig. 6). Near the juncture of the dense matrix of Schwann cell processes interdigitating with the ribosome-rich keratinocytes, the Schwann cytoplasm often displayed clusters of micropinocytotic vesicles. As the axon penetrated the epidermis it was accompanied by thin Schwann cell processes until it was completely enveloped by a basal keratinocyte (Fig. 7), a feature that is apparently absent in other receptor arrangements and was not regularly observed outside of the marked zone in the same specimens. Within the axons, at the site of epidermal penetration, there were sparse clusters of clear round or pleomorphic vesicles and occasional large dense-core vesicles, but these were rare after the axon–Schwann cell complex was enveloped within the epidermis (Fig. 7) or when the axon lost its Schwann sheath and was completely surrounded by keratinocytes (Fig. 8).

The intraepidermal axon is clearly never a free or bare nerve ending, nor is the nature of its receptive site ever certain. Whether any axons can be traced to more superficial layers is dubious (87), since no

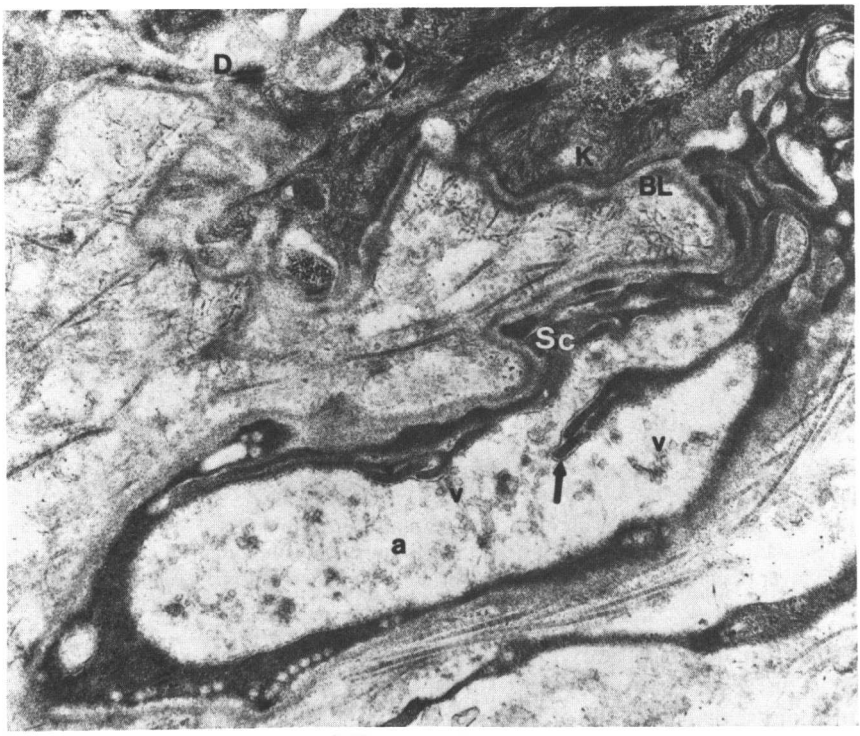

Fig. 6. Electron micrograph of epidermal penetration site of single unmyelinated axon (*a*) containing patches of clear vesicles (*v*) and a few dense-core vesicles, surrounded by Schwann cell processes (*Sc*) that appear to bifurcate (*arrow*) near the axon's contact with basal lamina (*BL*) of epidermal keratocyte (*K*), displaying typical desmosomal junction (*D*). Note fusion of basal laminae and Schwann cell pinocytotic vesicles in marked nociceptor spot. ×12,800. [From Kruger, Perl, and Sedivec (69).]

convincing examples of intraepidermal axons unassociated with Merkel cells have been observed in several decades of electron-microscopic study (11, 12). This is somewhat difficult to reconcile with silver-impregnated fibers, some having been traced into the stratum corneum with optical microscopy (Fig. 9; 41, 106). However, there is some recent evidence of deep intraepithelial axons in electron micrographs of the oral mucosa (Y. Yeh and M. R. Byers, personal communication), and it is conceivable that the widely spaced intraepidermal axons seen with metallic impregnation might correspond to nociceptor spots.

To date, in each example of morphological correlation, examination of a spotlike receptive field has revealed an axon penetrating the epidermal basal lamina; this now appears to be the rule for Merkel disks (57), cold spots (47), and myelinated nociceptors (69). Because most other afferent fibers display broadly distributed receptive fields,