Spinal Neurons Specifically Excited by Noxious or Thermal Stimuli: Marginal Zone of the Dorsal Horn

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HIGH-THRESHOLD MECHANOSENSORY UNITS and their centrally projecting myelinated fibers make up a functionally distinct group of cutaneous sensory units that have been suggested as part of the afferent apparatus for pain resulting from mechanical damage to the skin (3, 17). The argument for their relation to pain was based on two points: 1) the ability of such afferent elements, unique among those with medullated fibers, to provide signals differentiating noxious from innocuous mechanical events affecting the skin; and 2) the well-established correlation between pain and activity in thin myelinated afferent fibers (7, 12). Nonetheless, a convincing case for the functional significance of sensory structures in so diversified a tissue as the skin requires additional evidence. If the high-threshold receptors have no connection to central neurons independent of more sensitive receptors, they would appear to be constituents of systems with no absolute specificity for the intensity of stimuli. On the other hand, if certain central cells receive a sole or major excitatory drive from high-threshold sense organs, it would suggest that such receptors are truly the first stage of mechanisms specialized for signaling marked distortion or damage of tissue.

The present work was a search for projections of the high-threshold mechanoreceptors to spinal neurons. The experimental design depended on the observation that in certain nerves supplying the cat's hairy skin, all or nearly all of the myelinated afferent fibers conducting under 30 m/sec are connected to either high-threshold mechanoreceptors or to very sensitive hair receptors (3). Therefore, to demonstrate that excitation of a central cell is dependent on an input from the high-threshold mechanoreceptors, it would be necessary to show that activity in slowly conducting myelinated fibers was required, that gentle mechanical stimuli were ineffectual, and that responses could be evoked by intense mechanical stimulation of the skin. Using this approach, a major excitatory connection from these high-threshold primary afferent units was found to the relatively large cells in the most superficial layer of the spinal cord dorsal gray matter. In addition, certain cells, similarly located, were shown to be activated by nociceptors or low-threshold thermal receptors with unmyelinated afferent fibers. These results were taken to indicate that the dorsal horn marginal layer, Rexed's lamina I (20), represents a specialized sensory nucleus containing neurons important for nociception and for detection of thermal changes in the skin. A preliminary report of some of the material has been presented elsewhere (6).

METHODS

Results are described from experiments on 52 adult cats made spinal by transection of the spinal cord at the upper cervical level under diethyl ether anesthesia. After spinal section, the preparations were artificially resired, all of the rostral brain destroyed by occlusion of its circulation, and further anesthesia discontinued. Systemic arterial pressure (mean levels of 60 to 100 mm Hg were considered acceptable) and end-tidal CO₂ (3-5% were considered acceptable) were continuously monitored. Since the dorsal horn responses were found to be susceptible to general deterioration of the preparation, data from experiments in which the mean arterial pressure was below 50 mm Hg and end-tidal CO₂ above 5.5 or under 3% were discarded. Rectal temperature was main-
tained at 37°C by automatic control of external heat. Bilateral pneumothorax openings were usually made to reduce spinal cord movement with respiration.

A laminectomy exposed the sacral and coccygeal spinal cord. The posterior femoral cutaneous nerve or a coccygeal (caudal) dorsal root was freed from surrounding tissue and placed on a bipolar stimulating electrode distally and a bipolar recording electrode centrally, leaving both peripheral and central connections intact. Neural tissue was prevented from drying by a cover of mineral oil in pools formed from skin and muscle flaps. The coccygeal dorsal roots innervate deep tissue of the tail as well as skin, but most of their fine myelinated afferent fibers are of cutaneous origin so that, in effect, they largely represent a cutaneous input for this portion of their spectrum. An approximate outline of the cutaneous field supplied by afferent fibers in the ncrvc or root was obtained (and marked with washable ink) by listening to the amplified discharges from the gross recording electrode while gentle mechanical stimuli were applied to the skin. The shock intensities necessary to excite either all of the myelinated afferent fibers of the bundle or just those conducting over 30 m/sec were determined by recording compound action potentials with the centrally placed pair of electrodes. The stimulator output could be switched between these two intensities during the course of the experiment.

Recordings from the spinal cord were made using glass pipette electrodes drawn to tip diameters of 2 μ or smaller. Electrodes were usually filled with 2–3 M NaCl and a saturated solution of dye (fast green FCF, C. I. 42053). Electrode impedances at 1,000 Hz ranged between 5 and 30 megohms. The microelectrode was advanced into the tissue 1 μ at a time by a hydraulic manipulator driven by a stepping motor. The microelectrode was connected to a low-capacity relay that could be switched to either a unity-gain impedance-matching amplifier or a source of direct current (23). After concluding an observation of interest, the recording electrode was made negative and dye extruded electrophoretically from it by passing a current of 10 15 μA for 5 min. At the termination of the experiment, usually within 2 hr after the first dye mark had been made, the animal was perfused through the aorta with a solution of 15% formalin in water. Frozen sections at 20 μ were made from the spinal cord regions of the microelectrode penetrations. In most instances, dye marks were first located in unstained sections prior to permanent mounting and a sketch or photograph of their positions made. Sections containing the dye marks were mounted and counterstained, either with thionin or cresyl violet. The positions of dye marks relative to other landmarks were determined from microscopic examination of the stained material.

Skin stimuli were delivered using the various devices described in previous publications (1, 3). During the course of the experiment, the amplified potentials from the monitoring electrode on the nerve trunk, from the microelectrode inserted into the spinal cord, and from sensors measuring stimulus intensity were displayed on different channels of an oscilloscope. The amplified microelectrode recording was also fed to an auditory monitor. Permanent recordings for these variables were made on analogue magnetic tape recordings using an FM system. Analogue reproductions of the signals by ultraviolet oscillograph were used for preliminary study of the magnetic tape recordings. Detailed analyses of the response of individual cellular elements were performed after conversion of the analogue electrical records to digital form; discharge patterns were plotted with the aid of an interactive graphics system utilizing a digital computer (see Schmittroth in 1).

RESULTS

Specific response to activity in slowly conducting afferent fibers

At certain locations in the vicinity of the root entrance zone, the response recorded from the surface of the spinal cord varied with the composition of the afferent volleys. At the rostral limit of a dorsal root containing fibers excited by the electrical stimulus, a part of a negative wave evoked by a single afferent volley appeared only in association with activity of the smaller diameter myelinated fibers. The distinction between the responses to volleys made up of only "Aβ"-fiber activity (over 40 m/sec) and those including impulses in slower fibers was especially clear in the case of dorsal roots innervating the tail. The entrance zone for each segment at this level of the spinal cord is well defined and the entry of each rootlet can be easily distinguished. The portion of the response particularly associated with the slowly conducting myelinated fibers was most prominent in the vicinity of cephalad rootlet. Examples of the potentials recorded at the rostral pole of a coccygeal segment after volleys of different composition appear in Fig. 1; the responses in column A were evoked by a volley of impulses confined to rapidly.
conducting myelinated fibers and those of column B by a volley in both rapidly and slowly conducting myelinated fibers (the lower traces of A1 and B1 show the compound action potential recorded from the root for the two stimulus intensities).

The arrow in Fig. 1, column B, indicates the additional component that appeared at such locations in response to volleys containing δ-fiber activity (under 30 m/sec). At the level of the more caudally entering filaments of a given dorsal root the difference in response related to components of the afferent volleys was less marked. This distinction also decreased 2–3 mm cephalad to the most rostral rootlet containing fibers excited by the shock.

The component of the response associated with the slowly conducting myelinated fibers became more prominent as the microelectrode penetrated into the spinal cord substance. This is shown for the experiment of Fig. 1 by records 2 and 3, obtained from locations approximately 100 and 200 μ beneath the surface. Although variations appeared in the evoked potential as the microelectrode advanced into the cord, only the part designated by the arrow was dependent on impulses in slowly conducting myelinated fibers. Typically, as illustrated by Fig. 1 A4 and B4, the difference between the responses to volleys in α-fibers and those to volleys in α- and δ-fibers disappeared deeper (400–800 μ) within the spinal cord and the first portions of the response greatly increased in size (amplification for A4 and B4 is 40% of that in the upper records).

These features of the response recorded near the cord surface led us to concentrate on the more superficial layers of the dorsal gray matter and our sampling of activity was primarily from these regions dorsal to Rexed’s lamina V (20). Afferent volleys of the type that differentiated between components of the response of Fig. 1 were used in the search for unitary activity. During advancement of the microelectrode, volleys composed of impulses in all myelinated fibers were employed. From time to time, as penetration progressed, evoked unitary discharges were noted. These were routinely tested for characteristics of postsynaptic elements. Discharges were considered to be postsynaptic in origin if the latency of the first impulse evoked by single volleys at low repetition rates (1/sec) varied by more than 0.5 msec, if they failed to respond to each supra threshold stimulation at repetition rates between 1-50/sec, and if they showed substantial latency changes (1 msec or more) as the volley repetition rate was increased. Unmyelinated afferent fibers might exhibit the latter two features, but it is unlikely that stable recordings were obtained from them (unpublished observations). In practice, discharge from elements

FIG. 1. Responses to afferent volleys recorded with a microelectrode at the dorsal root entrance zone of a coccygeal segment. Volleys consisted of impulses in rapidly conducting myelinated (α) fibers only for column A and in both rapidly and slowly conducting myelinated fibers (α and δ) for column B. Compound action potentials recorded from the dorsal root 15 mm central to the stimulating electrode are shown by the second trace of horizontal row 1 (the late, low-amplitude irregular activity in these records represents dorsal root reflex in some rapidly conducting fibers). Two successive oscilloscope sweeps are superimposed for each microelectrode record (positivity up); amplification was the same for horizontal rows 1, 2, and 3 and 40% for 4. Row 1, spinal cord surface at the dorsolateral sulcus near the rostral pole of the segment; row 2, approximately 100 μ within the spinal cord; row 3, approximately 200 μ within the spinal cord; row 4, approximately 550 μ within the spinal cord. Arrows of column B indicate deflections typically related to activity in the slowly conducting myelinated fibers.
encountered in the regions explored posed little problem since their characteristics were unequivocally either those of primary myelinated afferent fibers or of neurons excited after synaptic transfer. The former's responses to suprathreshold stimuli varied in latency by 0.1 msec or less and an impulse appeared to each volley at repetition rates over 100/sec.

Postsynaptic unitary responses that depended on activity in slowly conducting afferent fibers were regularly seen, although they were not obtained in every penetration. By and large, units meeting this condition were found at depths where there was a prominent deflection in the responses evoked by single volleys of the type marked by the arrows in Fig. 1B. It was rare to record from more than one such unit at a given locus with electrodes over 10 megohms in impedance. Judging from the sequence of events during penetrations and the frequency of encountering these units, it was our impression that the cells generating the discharges were sparsely distributed within a limited region. In Fig. 2 the upper of each pair of traces is a compound action potential recorded from a coccygeal dorsal root and the lower trace the simultaneous microelectrode recording from within the spinal cord. An afferent volley containing a near maximal Aα component, and some of the Aδ impulses, did not evoke a response from the cell (Fig. 2A) that occasionally produced the large unitary potential after the addition of more δ-fiber activity to the volley (Fig. 2B). For the record of Fig. 2C the stimulus to the dorsal root was increased to a level maximal for all of the myelinated components; the five superimposed traces show that the cell discharged to each afferent volley with latency shifts of as much as 1 msec. The central delay for activation of such units was impossible to calculate accurately, because the exact conduction time of the excitatory fibers could not be determined. The delay within the spinal cord after arrival of impulses in fibers conducting under 40 m/sec varied from less than 1 msec to over 4 msec.

With rare exception, units, recorded deep to the level giving rise to the deflection related to the slowly conducting myelinated fibers, responded to volleys only in the most rapidly conducting fibers. Although these elements were not systematically studied, gentle mechanical stimulation of hairs or the skin excited those that were tested. The trajectory of electrode tracks and calculations of the depth of the electrode tips suggested that postsynaptic elements activated by rapidly conducting afferent fibers and gentle mechanical stimuli were located in lamina IV or deeper (see also 24, 25).

The following descriptions are based on data from 110 units whose excitation depended on afferent fibers conducting under 30–40 m/sec; 11 were recorded from the lumbosacral segments contributing to the posterior femoral cutaneous nerve and the remainder from coccygeal segments.

ELEMENTS RESPONDING ONLY TO INTENSE MECHANICAL STIMULATION OF SKIN. Units of the type illustrated by Fig. 2 did not respond to gentle mechanical stimuli and presumably received synaptic excitation from the afferent fibers of the high-threshold mechanoreceptors. This deduction was confirmed by the effects of the stimuli particularly effective in exciting such receptors: distortion of the skin produced either by pressure from sharply pointed objects or by firmly squeezing a skin fold between two rigid surfaces (3). The unit of Fig. 3 responded only to volleys in the more slowly conducting myelinated afferent fibers and was preferentially excited by noxious mechanical stimuli. Figure 3A depicts two stimulation periods during which the skin was firmly stroked with a smooth glass rod and only an occasional discharge was evoked; the high-threshold mechanoreceptors are weakly excited, if at all, by this

![Figure 2](http://jn.physiology.org/)

**Figure 2.** Unitary responses to afferent volleys of differing composition. Upper trace of each pair is the compound action potential recorded from the coccygeal dorsal root and the lower trace the microelectrode recording from within the spinal cord. Electrical shock in A sufficient to excite nearly all α-fibers (over 40 m/sec) and some δ-fibers (under 30 m/sec); B, shock intensity increased slightly over that in A; C, shock intensity maximal for all myelinated fibers (5 sweeps superimposed).
FIG. 3. Responses of a spinal neuron to mechanical stimulation of the skin. Upper trace of each pair is the microelectrode recording from a coccygeal segment and the lower trace marks the approximate time and duration of stimuli delivered to the skin of the tail. Unit gave responses linked to slowly conducting myelinated components of afferent volleys and was not excited by skin cooling or noxious heat. A, firm stroke with a smooth glass rod; B, squeeze of a fold of skin in the receptive field by a smooth surfaced forceps with greater pressure exerted for the second stimulation; C, squeeze of a fold of skin of the receptive field by a serrated forceps with pressure maintained for the stimulation period.

form of manipulation. Squeezing a fold of skin with a calibrated forceps exerting 100–150 g between two areas about 5 mm² consistently initiated responses (Fig. 3B). Pinching the same skin area with a serrated forceps caused a still greater discharge (Fig. 3C). The latter type of stimulus usually produces the most vigorous response from the high-threshold mechanoreceptors. Temperature sensitivity was tested by rapidly cooling the mechanoreceptive field 10°–20° C (with evaporation of ether or ethyl chloride) and by stepwise contact heating to 55 C. Neither the unit of Fig. 3 nor 33 similar units responded to these strong thermal stimuli. An additional 18 units were not adequately tested for one of the thermal changes but otherwise had characteristics suggesting that they belonged to this category. The selective responsiveness to marked mechanical distortion was taken as evidence for high threshold mechanoreceptors as the principal or exclusive source of excitatory drive. Additional support for this reasoning came from the fact that units of this category did not alter their response to afferent volleys when C-fiber components were added.

Background activity of spinal cells responding only to intense mechanical stimuli was rare and their evoked response, even to the strongest stimuli, showed little or no afterdischarge. Adaptation during maintained stimulation was consistently observed; however, some discharge was often present for several seconds. The graphic display of Fig. 4, derived from the response of Fig. 3C, illustrates this latter point for the highly noxious stimulus produced by maintained pressure across a fold of skin by a tissue forceps, the teeth of which penetrated the surface. The response consisted of a relatively high frequency burst of impulses at the beginning, followed by a gradual decrease in discharge rate. With waxing and waning, impulses continued to be generated for the duration of the stimulus at a low frequency (under 20/sec). The irregular discharge evident in Fig. 4 is representative of the response pattern displayed by this entire group of cells.

Receptive fields for the high-threshold mechanoreceptive units varied considerably in size. The excitatory zone was confined to a skin area ipsilateral to the recording site; on the tail it sometimes occupied a large part of one dermatome (4–5 cm long), although certain units could be excited only from a region of 1 cm². Variations in the effectiveness of equivalent stimuli applied to different portions of the receptive field were usually demonstrable. A centrally located portion of the field evoked the
greatest response from some units. Typically, use of punctate stimulators such as a needle or a stiff hair disclosed an uneven or spotty arrangement, particularly near the edges of the receptive field; at these locations responses could be elicited from one small area but not an adjacent one even with more intense stimulation. It was often difficult to determine whether stimulation of subcutaneous structures also excited a coccygeal unit because the skin was difficult to lift clear of deep tissue of the tail. In some cases, displacement of the excitatory field with skin movement suggested that only a cutaneous input was involved, whereas in others, deeply located receptors requiring strong stimulation either added to the response evoked from the skin or could initiate one by themselves.

It seemed likely that more than one primary afferent fiber converged on a given postsynaptic element. If an afferent volley was gradually changed in composition so as to include more and more primary afferent units, the latency of the unitary discharge progressively shortened and in some instances repetitive discharge appeared. Furthermore, some units seemed to have had excitatory skin fields that extended beyond the limits of one dermatome (see METHODS), implying that primary afferent elements of more than one root projected to a given cell. However, in several experiments electrical stimulation of dorsal roots adjacent to one driving a cell did not evoke a response. Since the sizes of primary afferent receptive fields on the tail have not been systematically studied, receptive-field dimensions could not be used as a measure of convergence in this region. Judging from studies on the posterior femoral cutaneous and sural nerves (3, 4), some high-threshold mechanoreceptors may have receptive terminals distributed over as large an area as that innervated by a coccygeal segment. On the other hand, receptive-field dimensions of those spinal units excited by posterior femoral cutaneous input were larger than those of the typical primary afferent units of this bodily region.

ELEMENTS EXCITED BY BOTH NOXIOUS MECHANICAL AND HEAT STIMULI. Fourteen dorsal horn neurons had features setting them apart from those just described while being similar in that their activation depended on slowly conducting afferent fibers and strong mechanical stimuli. Over one-half of this second group were found to give a double burst of impulses when an excitatory volley had a C-fiber component. The first response occurred shortly after the arrival at the spinal cord of impulses in δ-fibers, with the second discharge appearing 3–10 msec following those in C fibers. Some of these cells showed background discharge prior to any form of skin stimulation and almost all exhibited tonic activity following intense mechanical or heat stimuli.

Graphic representation of the responses of a unit excited by both slowly conducting myelinated and by unmyelinated fibers appears in Fig. 5. Only a few discharges were recorded from this element during squeeze of a skin fold by a smooth forceps applied with 100 g of force, whereas a more vigorous pinch elicited more impulses (Fig. 5A). When a sharp probe was used, a response of considerably greater magnitude occurred consisting of an initial burst, as the point penetrated the surface, followed by low-frequency activity interspaced with occasional bursts (Fig. 5B). Qualitatively, the mechanically induced responses of such units differed little from those described in the previous section, although on the average, the threshold for mechanical stimuli was lower and sustained discharge was more prominent.

The unit of Fig. 5 responded vigorously to noxious heat, as is shown in C. Background activity from this cell was represented by an occasional impulse; the first unequivocal response to elevated skin temperature appeared at the step between 40 and 45°C with a larger barrage to a subsequent step from 45 to 50°C. When the contact thermode temperature was raised above 55°C, the stimulated portion of the receptive field sometimes appeared to be inactivated in the manner attending the last temperature elevation of Fig. 5C. (The cell continued to respond briskly when other portions of the receptive field were subjected to temperatures in excess of 45°C.) In contrast to the regular and vigorous responses initiated by noxious heat, sudden
Discharge pattern (instantaneous frequency) of a spinal neuron in response to various strong cutaneous stimuli. Unit gave responses linked to both \( \delta \) myelinated and C-fiber components of afferent volleys. A: squeeze of a fold of skin with a smooth-surfaced, calibrated forceps. The force exerted is indicated by the continuous line on the lower portion of the graph. B: pressure with a sharp probe at one spot of the skin; the point penetrated and remained in the skin for the duration of the stimulation period (bar). C: localized heat to a restricted portion (2 mm diam) of the receptive field by a controlled-temperature thermode (solid line in lower portion of graph gives thermode temperature in °C).

Skin cooling, even by 10°–15°C, seldom initiated discharges from these units unless the skin had previously been exposed to noxious thermal (or, less frequently, mechanical) stimuli. Several units of this group were tested and found to respond moderately briskly to 1 M acetic acid placed on their receptive fields.

The receptive-field arrangement for units responsive to both strong mechanical and thermal stimuli was not notably different from that described for those responding only to mechanical stimuli. Mild damage of the excitatory receptive field, particularly by heat, often was followed by a decrease of threshold to subsequent stimulation. Variations in the effectiveness of equivalent stimuli at different loci were regularly observed, and in some instances mechanical but not heat stimulation of a given spot was found to excite a unit. Examinations for dissociation between the two stimuli were not routine, so its prevalence was not established.

Spinal units responding to strong mechanical stimuli and to noxious heat apparently received an excitatory input from both the high-threshold mechanoreceptors with myelinated afferent fibers (3) and the polymodal nociceptors with unmyelinated afferent fibers (1). This deduction was based on three facts: 1) responses were evoked by afferent volleys in myelinated fibers, 2) a second burst of activity occurred with volleys containing C-fiber activity at a latency consistent with excitation from them, and 3) the effective mechanical and thermal stimuli were those capable of activating these two kinds of high-threshold sensory units. Polymodal nociceptors have moderate to high thresholds for mechanical stimuli, regularly respond to noxious heating of the skin, are excited by irritant chemicals, and respond poorly to cooling unless the skin has been damaged. The tendency of this group of spinal elements to develop background discharge and to display lower thresholds for cutaneous stimuli after skin damage may be related to the input from the unmyelinated fibers inasmuch as polymodal nociceptors of the skin exhibit tonic activity following intensive stimulation and become more responsive following noxious heat (1).
Elements responding to innocuous thermal stimuli. Twenty-three units gave marked responses to one or another form of innocuous thermal stimulation. Some of these neurons discharged to volleys containing impulses in only myelinated fibers at a latency similar to that shown in Fig. 2. When volleys with C components were used, such units usually gave a delayed second response consistent with a second period of excitation from unmyelinated afferent fibers. Certain units did not exhibit the second burst of impulses related to C-fiber input until the repetition rate of the volleys was increased; this procedure was not regularly employed because repetition of the strong shocks required for activation of unmyelinated fibers led to conduction block of myelinated fibers. In some instances a unit was recognized from the presence of background discharge since an evoked response appeared only to volleys containing activity in C fibers. None of the units of this group responded to any form of gentle mechanical stimuli but most were excited by intense mechanical manipulation of the skin. A few of those markedly influenced by innocuous heating or cooling, with effective excitation only from the C-fiber component of volleys, were very difficult to activate by mechanical means.

The response of one unit to a prolonged but moderate lowering of the skin temperature produced by ether evaporation is shown by the graph of Fig. 6A. For the greater portion of the response the discharge rate was under 40/sec, but bursts were interspaced during which the "instantaneous frequency" rose to 100-200/sec. Some increase in resting discharge or the evocation of a response from such units followed temperature changes as small as 1°-2° C at the skin surface. Most of the elements encountered with these characteristics gave their greatest response to skin cooling, although several were seen in which the principal responses were related to warming. The sensitivity of this group to innocuous thermal changes and their excitation by impulses in C fibers suggested that they received a projection from specific thermoreceptors (cf. 13).

Elements responding to cooling often gave a response to noxious heat (i.e., above 45 C) as shown in Fig 6B for the unit of Fig. 6A. A few impulses appeared at thermode temperatures between 30 and 40 C (Fig. 6B); however, these probably represented the tonic discharge of this element that was evident when the skin surface was at neutral temperatures (taken to be between 33 and 39 C). Other units with a conspicuous sensitivity to cooling ceased tonic discharge (present during prolonged cooling or at neutral temperatures) when the cutaneous surface was slightly warmed. The effects of noxious heat are reminiscent of the "paradoxical" response of "cooling" thermoreceptors to high cutaneous temperatures (8).

The receptive fields of the thermally sensitive units were not systematically investigated. Cooling or heating stimuli were effective when applied to relatively small areas located within the confines of a dermatome in the tail area, but no study was made of possible overlap from one segment to another. Units excited by innocuous thermal stimuli were not studied in the earlier experiments of the series employing the posterior femoral cutaneous nerve since the emphasis then was exclusively on cells responding to impulses in myelinated fibers.

Afferent inhibition. The initial evidence for an inhibitory input to these various types of units came from observations on those with background or tonic activity. When stimuli were applied to skin regions immediately outside of the excitatory field, they were found to inhibit the ongoing discharge. Subsequently, inhibition from peripheral stimuli could be demonstrated in most of the cases in which a search was conducted for it. Since many units did not have background activity, study of inhibition often required tests on evoked responses to either electrical stimulation of a dorsal root or adequate skin stimulation. Only coccygeal units were examined for inhibition. The inhibitory skin area was immediately adjacent to the excitatory field and often was most prominent proximal to the excitatory region. In several cases stimulation of areas both proximal and distal to the excitatory field inhibited background discharge or depressed evoked activity. Thus, the receptive field may be organized in a "surround" fashion, although the medial-
lateral distribution of the inhibitory effect was not sufficiently examined to be certain that the inhibitory field completely encircled an excitatory one. In a number of instances the excitatory skin on the tail extended from the dorsal midline to the ventral midline and no effects from the opposite side could be demonstrated.

The stimulus most effectively inhibiting either evoked activity or background discharge appeared to be similar in nature to that exciting the unit in question. The best evidence in this regard was collected for units responsive solely to strong mechanical stimuli. For these elements the cutaneous stimulus causing inhibition was intense mechanical deformation of the skin; thermal or weaker mechanical stimuli produced a lesser effect, if any. The available observations suggest that an inhibitory projection from skin fields adjoining the excitatory area probably is a regular feature of the functional organization for the spinal neurons of the present report.

**Location of recording sites and the neurons involved**

A total of 50 marks made by dye deposited electrophoretically from the recording electrode were located in the histological material: this represented approximately two-thirds of the instances in which marking was attempted. The marks consisted of collections of green dye ranging in size from approximately 20 to 100 μm in diameter associated with a variable amount of tissue distortion. The size of the mark apparently depended on the duration of current passage and the time elapsing between dye deposition and perfusion of the animal. No systematic reason for the failure to recover marks was determined. In some cases it was apparent that enough current could not be passed through the recording electrode because of its high impedance and occasionally dye was not expelled even though the current had been sufficient. At other times dye was lost from the sections in the histological preparation.

Most of the described responses were recorded close to the dorsal surface, usually within 200–500 μm of the place where the microelectrode contacted the surface. On the other hand, sometimes the same types of units were more deeply located and occasionally they were found at two different levels of the same or adjacent penetrations. The position of the dye marks explained these observations. The marks were always located close to the curved margin between the dorsal gray matter and the surrounding fiber tracts as shown by Fig. 7. Each point in Fig. 7 is indicated on the outline of a typical coccygeal section in terms of its position relative to the gray matter-white matter boundary and to the margin of the spinal cord. Recording positions from the lumbosacral segments of the posterior

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**Fig. 6.** Discharge pattern (instantaneous frequency) of a spinal cell responding to innocuous cooling. Unit gave responses linked to both δ myelinated and to C-fiber components of afferent volleys. A: cooling of the receptive field by ether evaporation (skin surface temperature rapidly decreased by 10°–12° C). Bar marks the application time of ether. B: heating of a small area of skin (2 mm diam) by a controlled-temperature thermode (solid line in lower portion of graph gives thermode temperature in °C).
FIG. 7. Location of dye marks at sites where unitary responses specifically related to slowly conducting afferent fibers were found. Position of each mark is indicated relative to major landmarks on an outline drawing of the dorsal horn in a coccygeal spinal segment. A: units excited only by strong mechanical stimulation of the skin. B: units excited by both noxious heat and by strong mechanical stimulation of the skin. C: relative position of three units recorded in one experiment: 1, unit excited by innocuous cooling, strong mechanical stimuli and noxious heat; 2, unit excited by strong mechanical stimuli and noxious heat; 3, unit excited only by strong mechanical stimuli. D: all dye marks recovered for units of the type described in this report.

Femoral cutaneous input were located on the same outline using identical criteria. Figure 7D summarizes data for all of the marks recovered for unitary recordings fitting the functional features outlined earlier. Most marks were in the marginal zone, Rexed's lamina I (20), and very rarely in the gray substance as deep as superficial portions of the substantia gelatinosa (lamina II). A few marks were situated in the white matter immediately alongside the margin of the gray substance. Lamina I in the lower spinal cord caps laminae II and III and marked recording positions were located throughout its extent. A number of the microelectrode penetrations passed sequentially through laminae II and III and terminated somewhere deep within lamina IV but loci for units of the type described were never found in either lamina III or lamina IV. This relation of the responses to the most superficial layer of the dorsal horn became evident midway through the study and was repeatedly confirmed in later experiments aimed at determining the position of units with one or another of the specific input patterns.

No systematic difference was established for the location of elements with the differing functional properties. The lack of a correlation between unit characteristics and location is documented by Fig. 7 in A, B, and C. Figure 7A gives the distribution of marks made for units excited by slowly conducting myelinated fibers and intense mechanical stimuli only, and Fig. 7B shows the position of marks recovered from the recording sites of units excited by intense mechanical and by noxious heat stimuli. Many of the latter were shown to have an excitatory input from both slowly conducting myelinated and from unmyelinated primary afferent fibers. In one experiment three units with three different patterns of activation were studied, each recording locus marked and subsequently located histologically. Their relative positions are illustrated in Fig. 7C. Although there was some difference in the rostrocaudal and mediolateral location of the marks of Fig. 7C, all were situated just at the margin between the white substance and the cellular region of the dorsal horn.

As Fig. 7A emphasizes, units equivalent to Fig. 7C, point 3, were found scattered throughout the entire mediolateral dimension of lamina I. Our observations do not indicate how much of the rostrocaudal extent of a segment's marginal zone participates in the specific projections from small-diameter afferent fibers since the explorations concentrated on the rostral pole of a segment where the activity they evoked was most prominent. Therefore, these data do not exclude the possibility that similar elements are located elsewhere or that some difference in the rostral-caudal organization exists.

Responses from primary afferent fibers were commonly seen; they regularly consisted of a largely monophasic positive impulse (§). In contrast, the postsynaptic units forming the population under consideration were typically diphasic, often first appearing with a predominant negative phase and with time or advancement of the electrode becoming larger in amplitude and exhibiting a progressively more prominent
positive phase. Occasionally intracellular penetration occurred with typical resting potentials and a large amplitude, essentially monophasic, positive-going spike. These findings suggested that the activity was recorded from the vicinity of a neuronal cell body that generated the discharge. Further evidence on the morphological element giving rise to the impulses came from the kind of observation illustrated by Fig. 8. In this experiment two units were studied, dye extruded at the recording site of each, and subsequently located in the histologically prepared material. The mark indicated in Fig. 8 as A was made for a unit responding only to intense mechanical stimuli and volleys in slowly conducting myelinated fibers. Later in this experiment a second unit was studied, which responded to both intense mechanical stimuli and to innocuous cooling. The second unit was driven by volleys in δ-myelinated fibers but further studies could not be carried out because the element became unresponsive, apparently due to intracellular penetration and consequent damage. Dye at the recording locus for the second unit (Fig. 8, B) appeared to be confined to the ramifications of a large stellate-shaped cell at the ventral margin of the dorsal horn. Dye could be seen in processes of the cell in several plans of focus and seemed to lie entirely within traces of the cellular outline. The insert, b, on the right of Fig. 8, shows an enlargement of B, indicating how the region darkened by the dye is surrounded by the nuclei of glial cells.

The histological preparations indicated that whereas large marginal cells were relatively sparsely distributed in the coccygeal spinal cord, from one to five could be identified in each 20-μm section. Some had their somata in the white matter separated by a band of myelinated fibers from the main body of the gray substance. Others were noted to lie over 50 μ within the gray matter. It was always possible to find one or more such large cellular elements in the immediate vicinity (i.e., ± 50 μ) of one of the marked recording sites. Their limited number and scattered position fit well with our experience in encountering units related to slowly conducting afferent fibers in exploration of the dorsal horn. The position of the marks and the location of these cells on the curved edge of the caudal dorsal horn also helps to account for the variable depth relative to the dorsal spinal cord surface at which the unitary responses were found.

**FIG. 8.** Photomicrographs of a spinal cord section containing dye marks. Left: dorsal horn region with two dye-mark locations indicated. The approximate boundary between white and gray matter is shown by dotted line. A, site of unit responsive solely to strong mechanical stimuli. B, dye mark at site of unit responsive to innocuous cooling. Inset: b, higher magnification of mark B; histological examination of the section indicated that all dye lay within the outline of a single large cell.
DISCUSSION

Two major points emerge from the present work. One is based on the specific nature of the projection of nociceptors to certain spinal cells; the other follows from the location of those spinal neurons found to receive a major or exclusive input from slowly conducting afferent fibers.

One kind of primary sensory unit (receptor) with a myelinated afferent fiber is excited only by intense mechanical stimulation of the skin (3). The fact that certain spinal neurons responded only to the same kind of cutaneous stimuli is part of the logic used to conclude that these cells had effective excitatory connections solely from that population of receptors. Viewed in another way this means that the stimulus specificity exhibited by the cutaneous receptors is carried over to certain central cells. Even though stimuli capable of exciting the central neurons are similar in characteristics to those activating the cutaneous sense organs, some integration of sensory signals does take place in the pathway. The spinal neurons activated only by myelinated fibers and by intense mechanical stimuli show evidence of excitatory convergence from several primary afferent fibers and an inhibitory input from regions adjacent to the excitatory field. Thus, the extent of body surface capable of effecting change in a cell's activity, as well as the degree of effectiveness of any given region, has been modified in the transfer of signals from the primary afferent fibers. Spinal cells receiving an excitatory input from both the high-threshold mechanoreceptors with myelinated fibers and the polymodal nociceptors with unmyelinated fibers similarly retain a good measure of stimulus selectivity. These latter cells are promptly excited by a range of environmental changes rather than solely by intense mechanical stimuli, but in all cases appreciable increase of activity only follows noxious events. Those neurons receiving excitation from more than one kind of high-threshold receptor also have receptive fields in which excitatory and inhibitory zones adjoin one another, again suggesting that the topography of the effective input is an important facet of the functional organization at this level. Inasmuch as the ability of the primary afferent units to discriminate dangerous from innocuous stimuli is transferred to spinal cells, both the receptors and these central neurons must be considered part of specialized systems actuated by the threat or fact of tissue damage.

The functional place of the spinal elements responding to innocuous thermal changes is less easily appraised. Most of this group were vigorously excited by sudden cooling at innocuous temperature levels, but gave nearly equivalent responses to noxious heat or intense mechanical stimuli. These findings do not necessarily imply a nonspecific function. Some time ago von Frey (11) reported that mechanical stimulation of certain cutaneous spots in man causes the sensation of cold. Presumably, a sensory structure at such a point can be excited by mechanical stimulation, yet the central nervous system's interpretation of the event is the sensation of cold. One could use a parallel argument for the spinal cells in question. Cooling is the innocuous and would be the more common excitant of such cells; therefore, their increased activity may signify skin cooling even though other stimuli are capable of initiating it. In any case, a decision on whether the units responding to moderate cooling are a link in specific thermally sensitive pathways or whether they are part of a nonspecific system requires further study. On the other hand, some of the marginal zone units had a limited reactivity to mechanical stimulation and responded well to only one direction of cutaneous temperature change; the latter could be easily fit into mechanisms important for temperature sense.

The location of the spinal neurons that generated the unitary responses has implications for their place in sensory processes. Although the marginal cells (Waldeyer) have received only occasional attention, their presence as a distinct feature of the spinal dorsal horn has long been recognized (2, 5, 20). Several anatomical studies have pointed out that fine-diameter afferent fibers have a number of synaptic terminations close to the point of entry in the zone containing the somata and some of the dendritic processes of these cells (9, 18, 21, 22). Thus, it is likely that the cells of
Rexed's lamina I receive an input directly from dorsal root fibers. From the available morphological evidence these synaptic connections could be either excitatory or inhibitory; however, presumptive support for a direct excitatory connection comes from the prominence of this effect at the segment of entry. The fact that several types of small-diameter afferent fibers project to marginal cells in a fashion organized from the standpoint of receptor type and receptor location offers physiological evidence for considering this accumulation of large neurons as a specialized sensory nucleus. Furthermore, the specificity of their activation makes it crucial that the projections of these spinal cells be determined in turn so as to define their part in pain and temperature sensibility. Some evidence on this point does exist from earlier studies. In Golgi material, Cajal (5) described axons from marginal cells of the chick passing into the lateral column of the same side, and Pearson (16) reported that in the cat some efferent fibers from large postero-marginal cells entered the crossed ventrolateral tract. In their classic study, Ranson and von Hess (19) implicated superficial dorsal horn structures, the substantia gelatinosa, and Lissauer's tract in conduction of impulses related to pain; however, their results depended on the effects of lesions that could just as well have been related to damage of the marginal zone or its outflow. On the basis of retrograde degeneration following ventrolateral cordotomy, Foerster and Gagel (10) and Kuru (15) concluded that the large postomarginal cells contributed to the crossed ventrolateral tracts of man. Furthermore, the correlation between segments containing retrograde changes and the dermatomal distribution of defects in the pain and temperature sense of his subjects led Kuru (15) to deduce that an important part of the pain-temperature pathway originates from these cells. On the other hand, the more generally accepted view would have the spinothalamic pathways arising from more deeply located neurons (2). Regardless of the way that the present results might fit with older observations and concepts, they direct attention to the cells of the dorsal horn margin and argue that these neurons have an important place in the mechanisms and pathways of pain and temperature.

Spinal neurons other than those of the marginal zone certainly receive excitation from high-threshold or thermal receptors. Those that Kolmodin and Skoglund (14) found to be excited only by noxious stimulation of the cat hindlimb or tail were located either well within the dorsal horn or near to the midline; both their location and responses suggest populations other than the cells of lamina I. Finally, our experiments were not designed to examine the question of whether some marginal cells have characteristics other than those we described. Although it is true that units with differing responsive features seemed to be far removed from the marginal zone, the present explorations concentrated on regions with a conspicuous input from slowly conducting fibers so that the absence of other types of elements in the results is inconclusive. Thus, determination of the extent of the functional spectrum of lamina I cells awaits data from another experimental approach.

SUMMARY

In unanesthetized, "decapitate" cats, recordings from the dorsal horn with micro-electrodes were used to investigate the relation between activation of specific kinds of cutaneous sensory units (receptors) with thin afferent fibers and the excitation of spinal cord neurons. The receptor type exciting particular spinal cells was deduced from a combination of observations on the conduction velocity of the afferent fibers evoking unitary discharge and on the nature of skin stimuli exciting the element.

At the rostral pole of segments in the lumbosacral and coccygeal regions, a component of the field potential evoked by single volleys was associated with activity in slowly conducting myelinated fibers. This deflection became more prominent with penetration into the most superficial layers of the dorsal spinal gray matter; at such loci postsynaptic units activated only by slowly conducting myelinated fibers (A) and/or unmyelinated fibers (C) were regularly encountered. The configuration of action potentials recorded from the postsynaptic...
units suggested that they were generated by the somadendritic portions of neurons.

The population of units fitting these general criteria could be subdivided according to their excitatory input. One group responded in a way indicating an effective drive solely from the myelinated fibers of high-threshold mechanoreceptors (3); they responded only to strong or frankly noxious mechanical stimulation of the skin. A second group, excited by both myelinated and unmyelinated afferent fibers, required noxious heat or intense mechanical stimuli for activation; apparently they were subject to convergence from both the high-threshold mechanoreceptors with myelinated fibers and polymodal nociceptors with unmyelinated fibers (1). A third group also had excitatory convergence from myelinated and from unmyelinated afferent fibers but were responsive to innocuous temperature changes of the skin in addition to intense mechanical and noxious heat stimuli. All three groups of cells apparently had an inhibitory input from receptors similar to those making the excitatory connection. Inhibitory receptive fields were positioned adjoining excitatory skin areas.

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