# CLASSIFICATION OF CAT VENTROLATERAL SPINAL AXONS AND THEIR RESPONSE TO AN ITCH-PRODUCING STIMULUS (COWHAGE)

by

Jen Yu Wei

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SUPERVISORY COMMITTEE APPROVAL

of a dissertation submitted by

JEN YU WEI

This dissertation has been read by each member of the following supervisory committee and by majority vote has been found to be satisfactory.

Chair : Robert Tuckett, Ph.D.

Paul R. Burgess, Ph.D.

Carlos E. Eyzaguirre, M.D.

Kenneth W. Horch, Ph.D.

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# FINAL READING APPROVAL

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Date

Tuckett,
Chairperson, Supervisory Committee

Approved for the Major Department

Carlos Eyzaguirre, M.D.
Chairman / Dean

Approved for the Graduate Council

B. Gale Dick
Dean of The Graduate School

## ABSTRACT

Unit activity was recorded from 92 ventrolateral spinal axons in seven anemically decerebrated spinal cat preparations, using a microdissection technique. Axons were classified into four categories according to their responsiveness to mechanical stimuli applied to skin and/or other tissues: 1) low threshold spinal axons (5%), 2) wide dynamic range spinal axons (57%), 3) high threshold spinal axons (23%), and 4) an "other" category which included axons responding to mechanical stimulation applied to visceral or deep tissue and axons responding to changes in limb or tail position.

Sixty-one of the 92 spinal axons had an ongoing resting discharge. Hence, they were divided into three types on the basis of their resting discharge pattern: 1) units with no resting activity (34%), 2) units with intermittent resting discharge (23%), and 3) units having continuous resting discharge (43%). Consequently, each category of mechanically sensitive ventrolateral spinal axons was further subgrouped on the basis of resting discharge.

In general, the fields of low threshold spinal axons were large and bilateral; the fields of wide dynamic range spinal axons were intermediate in size and ipsilateral; and

the fields of high threshold spinal axons were small and contralateral.

The approximate location in the ventrolateral spinal white matter of different categories of spinal axons was mapped but no significant segregation was found.

A comparison was made between different categories of mechanically sensitive ventrolateral spinal axons to determine their sensitivity to the itch-producing stimulus, cowhage. The wide dynamic range spinal axons were significantly affected. Of 34 wide dynamic range units which were tested, 23% demonstrated a relatively high sensitivity to cowhage and hence might be regarded as pruritogen-responsive spinal axons. Sixty percent of the pruritogen-responsive units had an intermittent resting discharge pattern.

Cowhage was applied on 14 high threshold and three low threshold ventrolateral spinal axons but no significant effect was demonstrated.

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# PREFACE

The concept that itch is a distinct sensory modality has gained increasing support in the recent years. If true, then according to the specificity theory of sensation an itch-related channel must exist (Willis & Coggeshall, 1978). This channel should include a peripheral receptor that encodes the signal for pruritus, spinal cord processing circuits, spinal pathways, and the central information processing circuitry that produce the perception of itch. Although numerous sensory channels have been proposed and documented, comparatively little is known about the itch-related channel.

After completion of a study of itch-related cutaneous receptors, my attention has been focused on how itch-related signals are transmitted within the central nervous system. The main purpose of this study was to find the distribution of itch-related spinal axons in the spinal white matter. Because ventrolateral cordotomy has been reported to release the itch sensation (Banzet, 1927; Hyndman & Wolkin, 1943), it was reasonable to concentrate this search in the ventrolateral spinal white matter.

This dissertation is in two parts. In Part I, a commonly used classification scheme has been used to classify cat ventrolateral spinal axons into four categories. Based on this classification, Part II presents evidence that only wide

dynamic range ventrolateral spinal axons were significantly responsive to the classical itch-producing agent, cowhage. To my knowledge this is the first time that pruritogen-responsive spinal axons have been carefully documented in the central nervous system. To this end, a new technique developed in recent years by this investigator was used instead of microelectrode recording. It provides much better recording stability which was essential for the present study because of the necessity of the long length of recording time and of the mechanical manipulating of the preparation. Further experiments are planned to search the dorsolateral funiculus, dorsal and ventral columns for itch-related sensory pathways.

I would like to express my sincere thanks to the members of my supervisory committee Dr. R.P. Tuckett, P.R. Burgess, K.W. Horch, C.E. Eyzaguirre, and G.G. Krueger for their encouragement, scientific guidance and help in completing the work for this dissertation and for their valuable criticism of the manuscript. I also wish to express my appreciation to the faculty and staff of the Department of Physiology for their encouragement and support. A special thanks is extended to Dr. H.T. Chang and E. Shen at the Shanghai Brain Research Institute and Dr. J.C.C. Hwang at Hong Kong University for their valuable contributions to my career and for their encouraging me to apply to graduate school. I particularly want to deeply thank Dr. R.P. Tuckett for his help in preparing and critically reviewing this manuscript,

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# PART I: CLASSIFICATION OF CAT VENTROLATERAL SPINAL AXONS

# INTRODUCTION

After completion of a study on itch-related cutaneous receptors (Tuckett & Wei, 1987a, b), my interest has been focused on how itch-related signals are transmitted within the central nervous system. The white matter of the spinal cord is composed of a large number of both ascending and descending fibers which transmit information between peripheral and suprasegmental nervous systems. The spinal cord pathways responsible for the sensation of touch-pressure, flutter-vibration, pain, temperature, position sense and visceral sensation have been extensively investigated. The results have been summarized in the monograph of Willis and Coggeshall (1978).

The mechanism by which the signal for itch is transmitted within the spinal cord is poorly understood. Clues come from clinical studies. For instance, Banzet (1927) reported that a case of severe itching with kraurosis vulvae was completely relieved by ventrolateral cordotomy. Relief was maintained when the patient was last seen two and one half years after the operation. Hyndman and Wolkin (1943) tested the response of patients to the classical itch-producing substance cowhage (Mucuna pruriens) following unilateral (N=3) or bilateral ventrolateral cordotomy (N=7). Itch was not evoked in the analgesic zones but was definite

in normal areas. White and co-workers (1950) reported that after ventrolateral cordotomy, itch from a poison ivy dermatitis was abolished in the analgesic zone. They also reported that a case of agonizing itching associated with an intramedullary neoplasm of the cord was abolished following ventrolateral cordotomy. Later, Arthur and Shelley (1959), Graf (1960) and Taren and Kahn (1966) reported similar clinical observations. This clinical evidence suggests that the sensation of itch is mediated through the ventrolateral portion of the spinal white matter.

Knowledge of the functional organization of the ventrolateral spinal white matter can be traced back to 1878 when Gowers reported his observations of a case involving a unilateral spinal gunshot injury. He suggested that pain and temperature sensations are transmitted by axons in the ventrolateral spinal white matter. In 1905, Spiller reported a patient who had spinal tuberculomas that bilaterally dis rupted ventrolateral spinal white matter. The patient had lost pain and temperature sensation over the lower half of his body, but tactile sensibility was preserved (Spiller, 1905). This observation not only led to the first cordotomy for pain relief (Spiller & Martin, 1912) but also provided strong clinical evidence to support the concept that a spinothalamic tract in the ventrolateral spinal white matter conveys information related to pain (Collier & Buzzard, 1903; Mott, 1895; Thiele & Horsley, 1901).

Since that time, many clinical investigations have focused on precisely locating pain-related spinal tracts. This has been done by performing cordotomies 1) at different neural axis levels (Graf, 1960; Walker, 1942) or 2) in different incision depths (White, et al., 1956; White & Sweet, 1969) and by 3) stimulating the ventrolateral spinal white matter prior to the operation with a percutaneous cordotomy technique in awake patients (Mullen, et al., 1963; Mayer, Price & Becker, 1975). As a consequence of these investigations, a detailed description has emerged of the functional organization of ventrolateral spinal white matter in man. It has been found that 1) the pain-related pathway is not a compact bundle as suggested earlier (White et al., 1956). 2) There are other pain-related pathways located outside the ventrolateral spinal white matter that appear to be of lesser importance (White & Sweet, 1969). 3) Besides pain- and temperature-related pathways, there are pathways in the ventrolateral spinal white matter related to itch (Banzet, 1927; Hyndman & Wolkin, 1943; White, et al., 1950; Arthur & Shelley, 1959; Graf, 1960; Taren & Kahn, 1966), tactile sensibility (Noordenbos & Wall, 1976), visceral sensation (White & Sweet, 1969), and position sense (White, et al., 1950, Noordenbos & Wall, 1976).

The complexity of functional organization of ventrolateral spinal white matter has been further demonstrated in animal studies. For example, behavioral changes following interruption of the ventral quadrant in dog and monkey have

been studied (Cadwalader & Sweet, 1912; Vierck, et al., 1971). These results not only confirmed the clinical observations mentioned above but also provided additional evidence suggesting that in the monkey some ventrolateral spinal axons contribute to weight discrimination (De Vito, et al., 1964). Another study showed that besides the spinothalamic tract there is a spino-reticular tract that projects to the medial tegmental region of the pons and medulla. Evidence from this study suggested that the spino-reticular tract might subserve the motivational and affective dimensions of pain (Field & Anderson, 1976).

Although evidence to date favors the notion that ventrolateral spinal axons may be involved in transmitting information related to different sensory modalities, little is known about which axon population might convey itch-related information. Furthermore, in contrast with numerous papers on the pain-related spinothalamic tract cells, which were based on unit potential recording from the soma-dendritic region in the monkey spinal grey matter, comparatively few experimenters have directly recorded unit potential activity from ventrolateral spinal axons. Several authors have mentioned that technical difficulties have limited the application of microelectrodes for recording from spinal axons (Oscarsson, 1964; Trevino, et al., 1972; Holloway, et al., 1978), especially in cat (Fox, et al., 1980; Hancock, et al., 1975). For instance, using microelectrodes to record unit activity from spinal axons may lead to

recording instability such that "...the duration of data collection is usually shorter than with extracellular recordings from the soma-dendritic region" (Willis, et al., 1975). Moreover, it is difficult "to establish that the cell body (of the spinal axons) was located within the spinal cord rather than in the brain" (Applebaum, et al., 1975). Although the microdissection technique used in this study cannot be used for recording from the soma-dendritic region, it complements the microelectrode technique by providing stable recording conditions and unambiguous records when making unit recordings from spinal axons (Wei, 1981); hence, it will provide a useful tool with which to classify and gain a better understanding of the functional organization of cat ventrolateral spinal white matter.

Although attempts have been made to classify cat ventrolateral spinal axons (Fields, et al., 1970; Pomeranz, 1973), none has classified them on the basis of a most commonly used classification scheme (Willis, et al., 1974; Price, et al., 1978; Dubner & Bennett, 1983; Chung, et al., 1986). In order to find the distribution of pruritogen-responsive spinal axons in the cat ventrolateral funiculus, and compare these axons with those documented in the exist ing related literature, a commonly used classification method (Willis, et al., 1974) was used in this study to 1) classify the ventrolateral spinal axons; 2) present correlations between different types of axons and the size and the location of their receptive fields; and 3) describe the

distribution of different axon categories in the ventrolateral funiculus. In the second paper (PART II) the sensitivity of different neuron categories to the itch-producing stimulus, cowhage, will be presented.

## MATERIALS AND METHODS

### Animal preparation and the dissection of spinal axons

To prevent the complications of both inhibitory and excitatory descending control (Jones & Gebhart, 1986; Soja & Sinclair, 1983; Tattersall, et al., 1986) and the effects of barbiturate anesthetic (Fields, et al., 1970; Raja, et al., 1986), cats were spinalized under Ketamine induced anesthesia (25 mg/kg + 0.1 mg Atropine i.m.) at the cervicomedullary junction and anemically decerebrated. Laminectomy was performed from L2 through L6. The dissection of spinal white matter was begun about 4-6 hours later to allow for recovery from spinal shock. Unit action potentials were recorded from filaments isolated at levels L2 to L3 of the ventrolateral funiculus by a microdissection technique (Wei, 1981; Wei, et al., 1984). The procedure for recording from ascending spinal axons was to pick up a strand of white matter from the rostral end of a small opening in the meninges with a pair of forceps. With another pair of forceps in the other hand, the strand was pinched in two at its most rostral point and then pulled gently away from the adjacent white matter and placed on a monopolar electrode of fine platinum wire 30 u in diameter. Because it is interrupted centrally, the

electrical activity recorded from this isolated filament must be from an ascending spinal axon.

There were two reasons for recording at L2 to L3 spinal levels. Making the recording site distant from synaptic regions insured 1) that the information transmitted through spinal cord relays was not distorted as a consequence of the dissection (Brown, et al., 1974; Carstens & Trevino, 1978) and 2) that the long ascending spinal axons from the hind limb had been formed (Ekholm, 1967).

Percent expired $CO_2$ and systemic arterial blood pressure were monitored. Data were collected only if these vital signs remained within normal limits (4% to 5%, and >70 mm Hg, respectively). Rectal and cord temperatures were maintained near 37° C with external heat.

The explored area

Histologically, lateral and ventral columns are separated by a line corresponding to the dorsal-most ventral roots (Beusekom, 1955; Chung & Coggeshall, 1983a). Division of the lateral columns into dorsolateral and ventrolateral funiculi can be defined by a horizontal line extending laterally from the intermediate horn (Beusekom, 1955). During an experiment it was possible to find the dorsal-most ventral roots on the ventrolateral surface of the cord, but a horizontal line cannot be drawn from the intermediate horn without spinal transection. Moreover, the location of the intermediate horn in different spinal segments varies considerably (Beusekom, 1955). For example, Rexed lamina VI

exists only in the cervical and lumbosacral enlargement of the spinal cord (Rexed, 1954). Therefore, the lateral denticular ligament was used as a landmark for division of the lateral columns into dorsolateral and ventrolateral funiculi. Hence, the area shown in the left side of Figure 1B can be considered to include one third to two thirds of the lateral part of ventrolateral funiculus and some of the most dorsal part of ventral column.

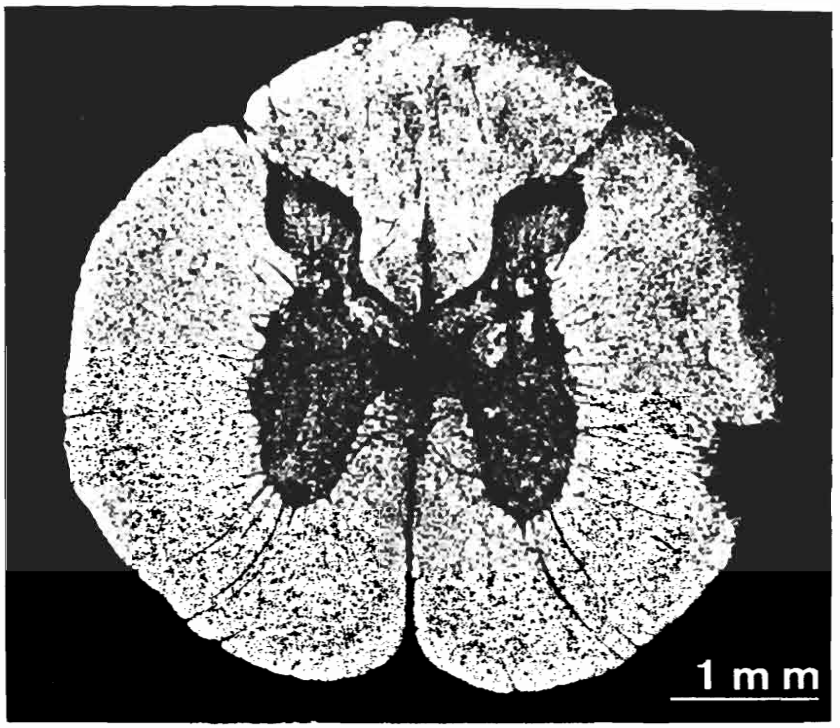
According to Flatau's law, long ascending and descending fibers course in the peripheral boundaries of the cord, whereas the shorter fibers are positioned more centrally (see Beusekom, 1955). Verhaart in his analysis of fibers in the cat anterior and lateral funiculi confirmed Flatau's law and noticed that the size of longer tracts decreased as they coursed caudally (Verhaart, 1953). Therefore, it seems reasonable to consider that the explored area (Fig. 1B) included mostly long spinal axons in addition to some propriospinal fibers (Chung & Coggeshall, 1983b). Because the present interest is to study the functional organization of the ventrolateral funiculus, detailed information on the origin and destination of composed axons has not been elucidated.

## Mechanical stimuli

All units described in this paper were activated with mechanical search stimuli delivered to the skin and/or deep tissue. In order to locate receptive fields and classify the units, different intensities of mechanical stimuli were used

Figure 1. Explored area in the cat ventrolateral spinal white matter. Panel A is a photograph of an 20 u thick unstained spinal cord section at L2, which was cut from a frozen block of tissue. The portion removed from the right side was the area explored during microdissection recording in one experimental animal. The right side of panel B has tracings from serial sections superimposed to show the outer perimeter of the cavity produced by the dissection, and the outline area on the left side represents the total range of explored areas from these seven experiments.

A

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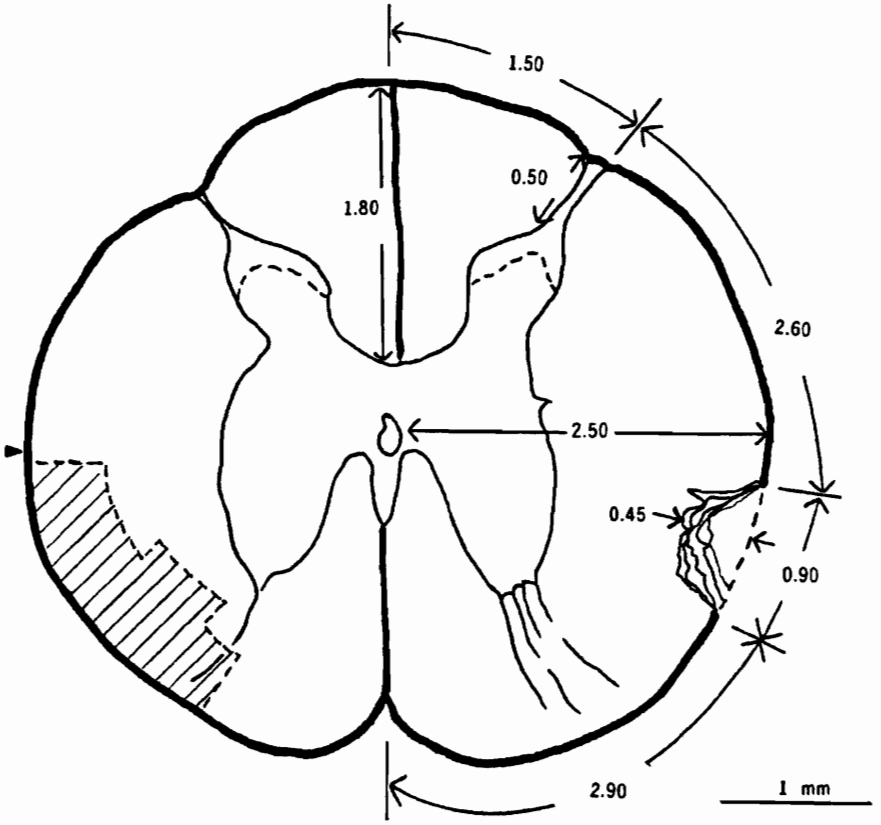


which included: 1) light brushing (LB) during which only the tip of a camel's hair brush touched the skin, 2) firm brushing (FB) for which the camel's hair brush was firmly pressed against the skin, 3) pointed probe pressing (P) during which a pointed hand-held probe (0.15 mm, O.D.) was pressed to the receptive field with a force of about 10 to 20g and 4) forceps pinching (F) for which a thumb-dressing forceps (with a tip area of 15 $mm^2$) was used to pinch the skin with a force of about 900g. When these stimuli were applied to the skin of the experimenter, light brushing induced the feeling of touch, firm brushing produced heavy pressure, and both pointed probe pressing and forceps pinching caused painful sensations.

Receptive field searches were limited to the hind limb and lower lumbar regions with no effort made to search for receptive fields on the upper part of the body. The portion of the field with the lowest threshold to mechanical stimulation was called the main receptive field (Chung, et al., 1986; Dubner & Bennett, 1983). Areas in which inhibitory effects could be induced by mechanical stimulation were designated inhibitory receptive fields (Chung, et al., 1986; Dubner & Bennett, 1983). The term "receptive field" appear ing in the following paragraphs will refer to the main receptive field unless otherwise specified.

## Histology

To measure the dissected area, the spinal cord was fixed in 10% formaldehyde. Twenty to 40 u thick serial frozen

sections were made. The outline of the gray and white matter including the dissected area was traced on paper with a Tri-Simplex microprojection (magnification 35X). The tracings were superimposed to obtain a drawing of the outer perimeter of the cavity produced by the dissection (the right side of Fig. 1B; Wei, 1981; Wei, et al., 1984). In some cases a photographic record of the dissected area (Fig. 1A) was obtained by placing the unstained sections on a glass slide and placing the slide directly in a photographic enlarger.

Because the filaments were dissected consecutively one after another and layer-by-layer in an ordered sequence, the relative location of each unit in the dissected area (Fig. 8) could be determined by the sequence number of the filament from which the unit was recorded. This method has been used to estimate the location of position-signaling and other types of spinal axons in the dorsolateral funiculus (Wei, et al., 1984).

Data recording and analysis

Neural signals were amplified with conventional electrophysiological instruments and stored on magnetic tape. For analysis, the tape was played back. Unit potentials were distinguished by a window discriminator which in turn gene rated a 300 usecond standard pulse for each discriminated action potential. The standard pulse was then sent to a computer and stored on diskettes as instantaneous frequency versus time or impulse frequency versus time histogram data.

Display and analysis programs were used for data manipulation such as setting the start and end times, normalizing, averaging and summing.

Before an experiment trial, the resting activity of every unit with ongoing discharge was recorded for one to four minutes. The resting discharge pattern of each unit was analyzed based on a histogram of impulse frequency versus time with a one second bin width that displayed data over a one minute period.

In order to compare the responses of different units to different intensities of mechanical stimuli, the response magnitude to a particular stimulus for a particular unit was evaluated as an averaged value (in impulses per second) calculated from the total number of impulses generated by the stimulus divided by the duration of the stimulus.

Statistical tests were run on a personal computer. Both the t test and a nonparametric analog of the t test, the Mann-Whitney two sample (MW) test, were used to evaluate whether two samples had been drawn from the same population. The nonparametric analog of the parametric one way analysis of variance F-test, the Kruskal-Wallis (KW) test, was used to decide whether k independent samples were from different populations (Hintze, 1986). Linear regression and correlation were used to evaluate the extent of relationship between two sets of data. In some cases, the normality of the sample distribution was tested with the method of moment or W-test (Yang, 1985).

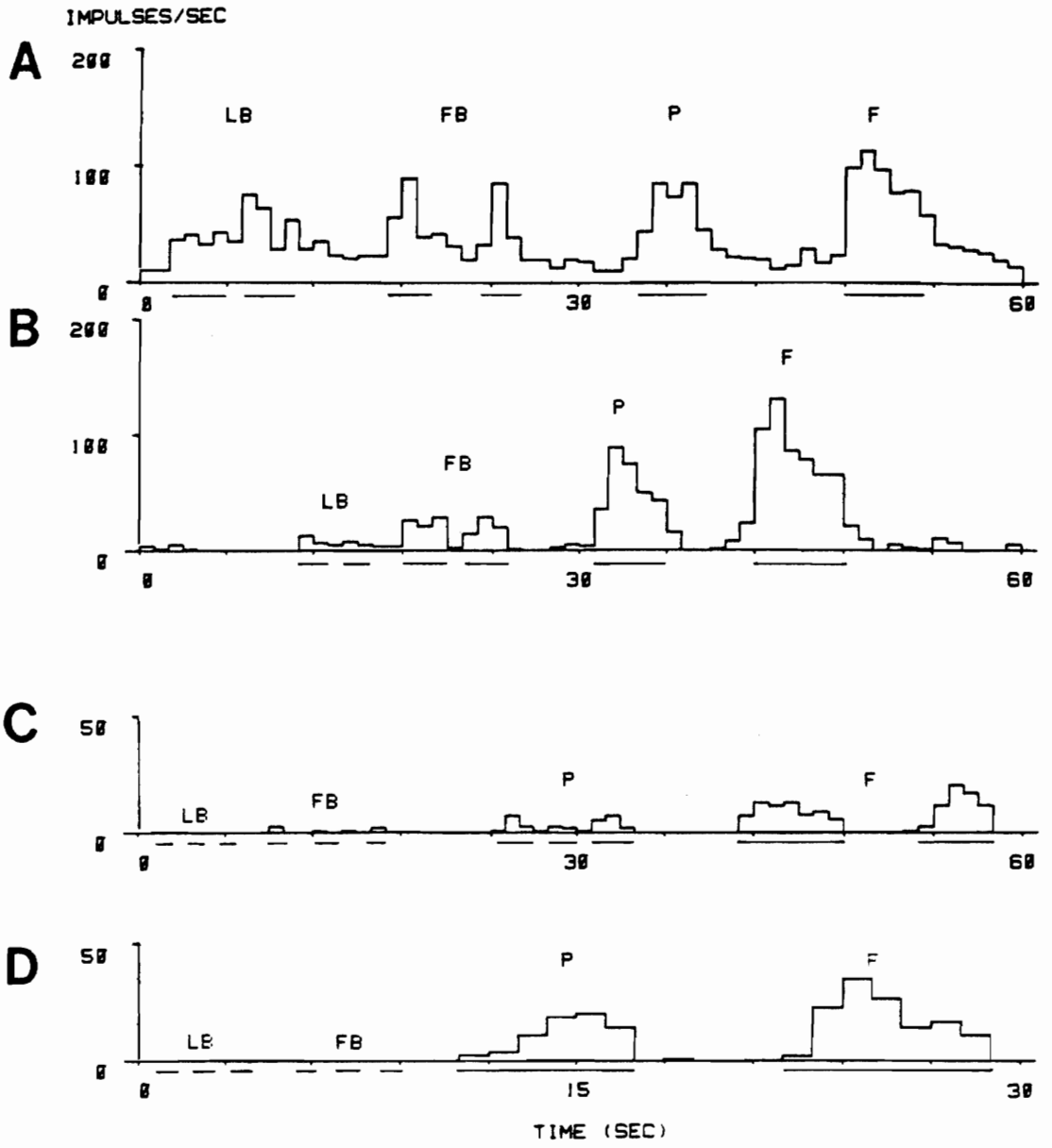
# RESULTS

## Response to mechanical stimulation

Ninety-two ventrolateral spinal axons from seven experiments, which could be activated by mechanical search stimuli, are presented in this paper. These axons were classified into four categories on the basis of their responsiveness to different intensities of mechanical stimuli as described in the materials and methods section.

Ventrolateral spinal axons which responded to innocuous mechanical stimuli, such as light brushing (LB) and firm brushing (FB), with the approximately the same response magnitudes as to noxious mechanical stimuli, such as pointed probe pressing (P) and forceps pinching (F), were categorized as low threshold spinal axons (Fig. 2A). Ventrolateral spinal axons responding to both innocuous and noxious stimuli in a graded fashion were called wide dynamic range spinal axons (Fig. 2B). Ventrolateral spinal axons which did not respond to light brushing were classified as high threshold spinal axons (Price & Dubner, 1977). About half of the high threshold spinal axons were driven by both firm brushing and noxious (P and F) levels of mechanical stimulation (Fig. 2C, Price & Dubner, 1977); the other half could only be excited by overtly noxious stimuli (Fig. 2D). Axons that responded

Figure 2. Impulse frequency versus time histograms illustrating the responses of different spinal axon categories to a range of intensities of mechanical stimuli. Panel A is a low threshold unit; B, wide dynamic range unit; C, high threshold unit which could be activated by firm brushing; D, high threshold unit which could only be excited by noxious mechanical stimuli. Abbreviations: LB, light brushing and FB, firm brushing with a camel's hair brush; P, pointed probe pressing; and F, forceps pinching. These abbreviations are used in subsequent figures. Bin width is 1 second. The horizontal lines under the histogram indicate the time during which the stimulation was applied. Note the the Y scale in panels A and B is larger than in C and D, and the X scale in panel D is smaller than in A, B, and C.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TIME (SEC)

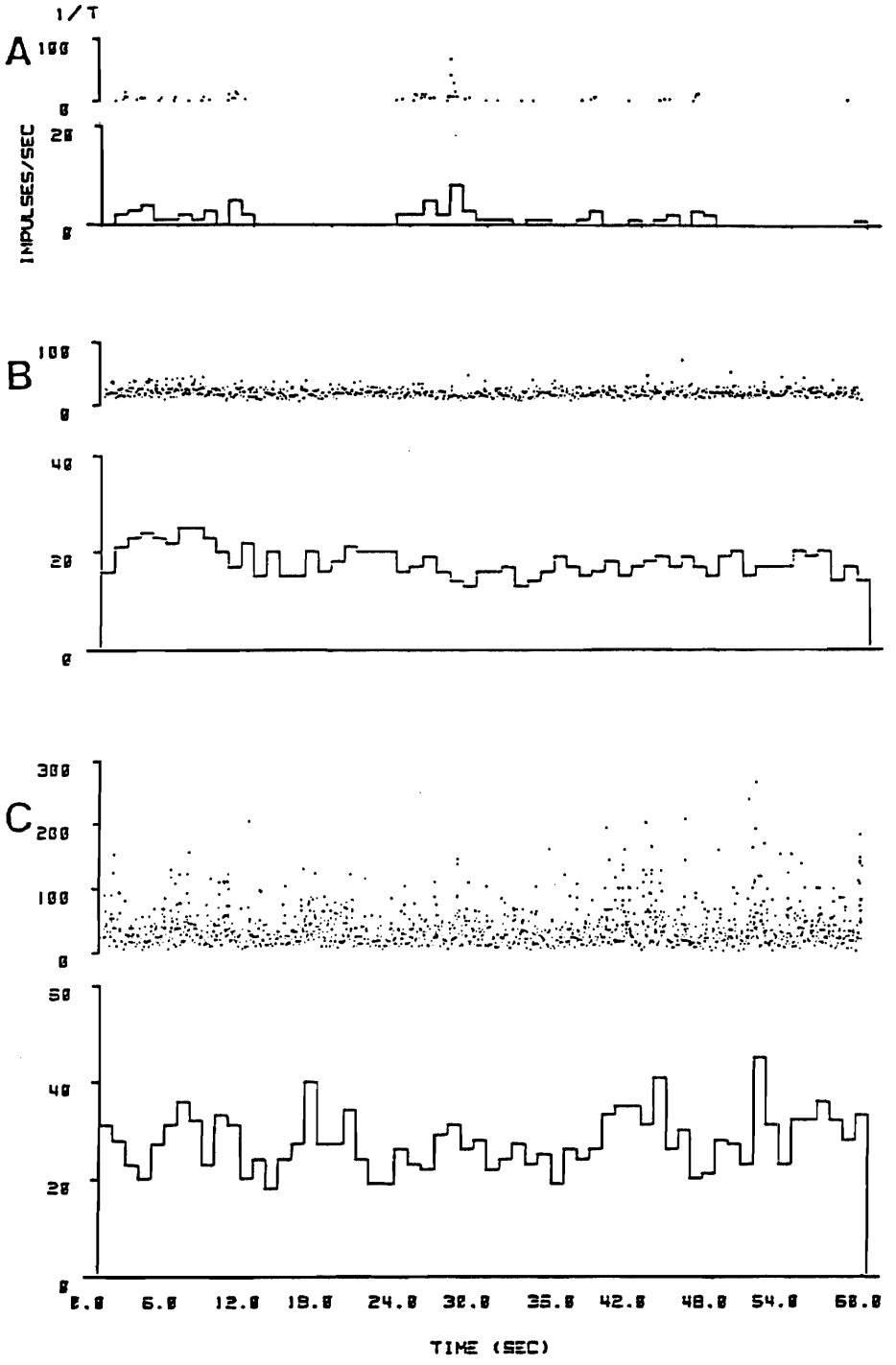
only to mechanical stimuli applied to viscera or deep tissues, or to changes in limb or tail position with no clear cutaneous innervation were grouped in an "other" category. Because this paper focuses only on the spinal axon population that was driven by cutaneous afferents, the "other" category was not considered.

Resting discharge pattern

Sixty-one of the 92 mechanically sensitive ventrolateral spinal axons demonstrated ongoing resting discharge. Hence, these axons could also be grouped into three types according to their resting discharge characteristics: 1) ventrolateral spinal axons with no resting activity, 2) spinal axons with intermittent resting discharge (Fig. 3A), and 3) spinal axons with continuous resting discharge (Fig. 3B & 3C). Within the continuous resting discharge population there was a range of variability in discharge. About half of the continuous resting discharge population showed a more regular resting discharge pattern (Fig. 3B) than the other half (Fig. 3C).

The definition of intermittent resting discharge was that during a one minute recording period there was at least one second during which no action potentials were recorded. Otherwise, it was called a unit with continuous resting discharge. Since the impulse frequency versus time histogram displays used a one second bin width, all histograms of units with intermittent resting discharge had at least one out of 60 bins in which the impulse frequency dropped to zero (Fig. 3A). The sample size of intermittent resting discharge units

Figure 3. Units with intermittent (A) and continuous (B & C) resting discharge patterns are shown as instantaneous frequency, reciprocal of interspike interval, versus time (upper trace) and as the corresponding impulse frequency versus time histograms (one second bin width, lower trace). In panel A, there are 31 bins with no activity indicating an intermittent resting discharge pattern. Panel B illustrates a unit with more regular discharge than unit in C. Note that the Y scale in panels A and B is smaller than in C.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42.0
48.0
54.0
60.0
TIME (SEC)

was 21 (Table 1). Therefore, subtracting the two units belonging to the "other" category left a remainder of 19. Of these 19 units, 18 complete records were obtained. The number of bins with no activity ranged from two to 57 (N=18, mean=25.7, SD=17.2, median=21.5) and was normally distributed (W-test, $p > 0.9$).

The total number of units with continuous resting activity was 40 (Table 1). After subtracting eight units belonging to the "other" category and three units with a resting discharge of less than one minute, complete records from 29 units were available. Impulse frequency vs time histograms of 18 units with intermittent resting discharge were summed, as were those of 29 units with continuous resting discharge (Fig. 4A & 4B). To match the sample size, 18 out of the 29 units with continuous resting discharge (Fig. 4B) were randomly selected and their resting discharge histograms were summed (Fig. 4C).

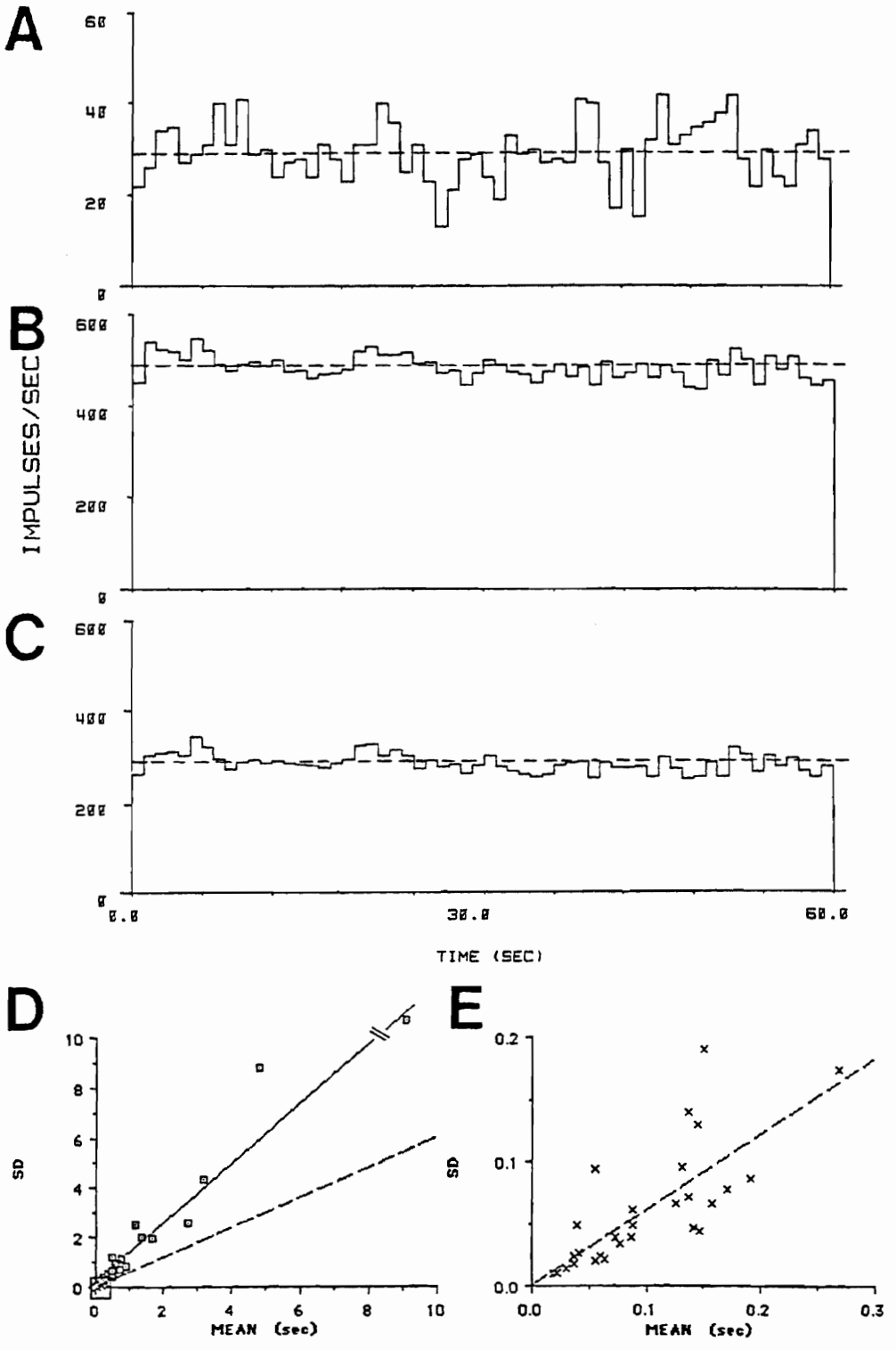
As a population, the distribution of fluctuations in discharge rate of both types (Fig. 4A & 4C) did not differ significantly from a normal distribution (method of moment test Fig. 4B, Ug1 $p > 0.5$, Ug2 $p > 0.5$; Fig. 4C, Ug1 $p > 0.2$, Ug2 $p > 0.5$). However, they did differ in two ways: 1) the average discharge rate of the summed histogram of 18 con tinuous resting discharge units (the broken line in Fig. 4C, mean=283.10, SD=21.08, N=60) was significantly greater than that of 18 units with intermittent resting discharge (the broken line in Fig. 4B, mean=29.38, SD=6.49, N=60, t test, $p <$

TABLE 1. Numbers and percentages of different spinal axon categories categories and subtypes.

| CATEGORY | Unit with no discharge | Unit with intermittent resting discharge | Unit with continuous resting discharge | Total | % |
|---|---|---|---|---|---|
| LT | 1 | 0 | 4 | 5 | 5 |
| WDR | 13 | 16 | 23 | 52 | 57 |
| HT | 13 | 3 | 5 | 21 | 23 |
| "Other" | 4 | 2 | 8 | 14 | 15 |

Explanation of abbreviations and headings : LT, low threshold spinal axon; WDR, wide dynamic range spinal axon; HT, high threshold spinal axon; "Other," including axons which respond to deep tissues or visceral mechenical stimulation, or to limb or tail position change.

Figure 4. Comparison of units with intermittent and continuous resting discharge patterns. Panel A shows a summation of impulse frequency vs time histograms of units with intermittent resting discharge (N=18, mean=29.38, SD=6.49, total spike count=1763). B shows units with continuous resting discharge (N=29, mean= 486.45, SD=25.79, total spike count=29187). C shows 18 units with continuous resting discharge which were randomly selected from B (N=18, mean=283.10, SD=21.08, total spike count=16986). The horizontal broken lines in panel A, B and C indicate average firing rates. D is a plot of standard deviation vs mean inter-spike interval of 18 intermittent (square) and 29 continuous resting discharge units (cross). For both plots the correlations were significant (for the intermittent resting discharge units r=0.98; for the continuous resting discharge units r=0.77; t test, $p < 0.001$). The solid line is the linear regression line for the intermittent resting discharge (slope=1.2, intercept=0.27) and the broken line, for the continuous resting discharge (slope=0.61, intercept=0.0012) units. The slopes of these linear regression lines differed significantly (U test, $p < 0.01$). A small square located at the upper right corner in D represents an off scale unit with a coordinate of X=16.31 Y=18.94. E is an enlargement of a large square area at the lower left corner of D showing the detailed scatter plots of units with continuous resting discharge patterns. Note the Y scale in panel A is ten times smaller than in B and C.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60.0
TIME (SEC)
SD
10
8
6
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2
0
0
2
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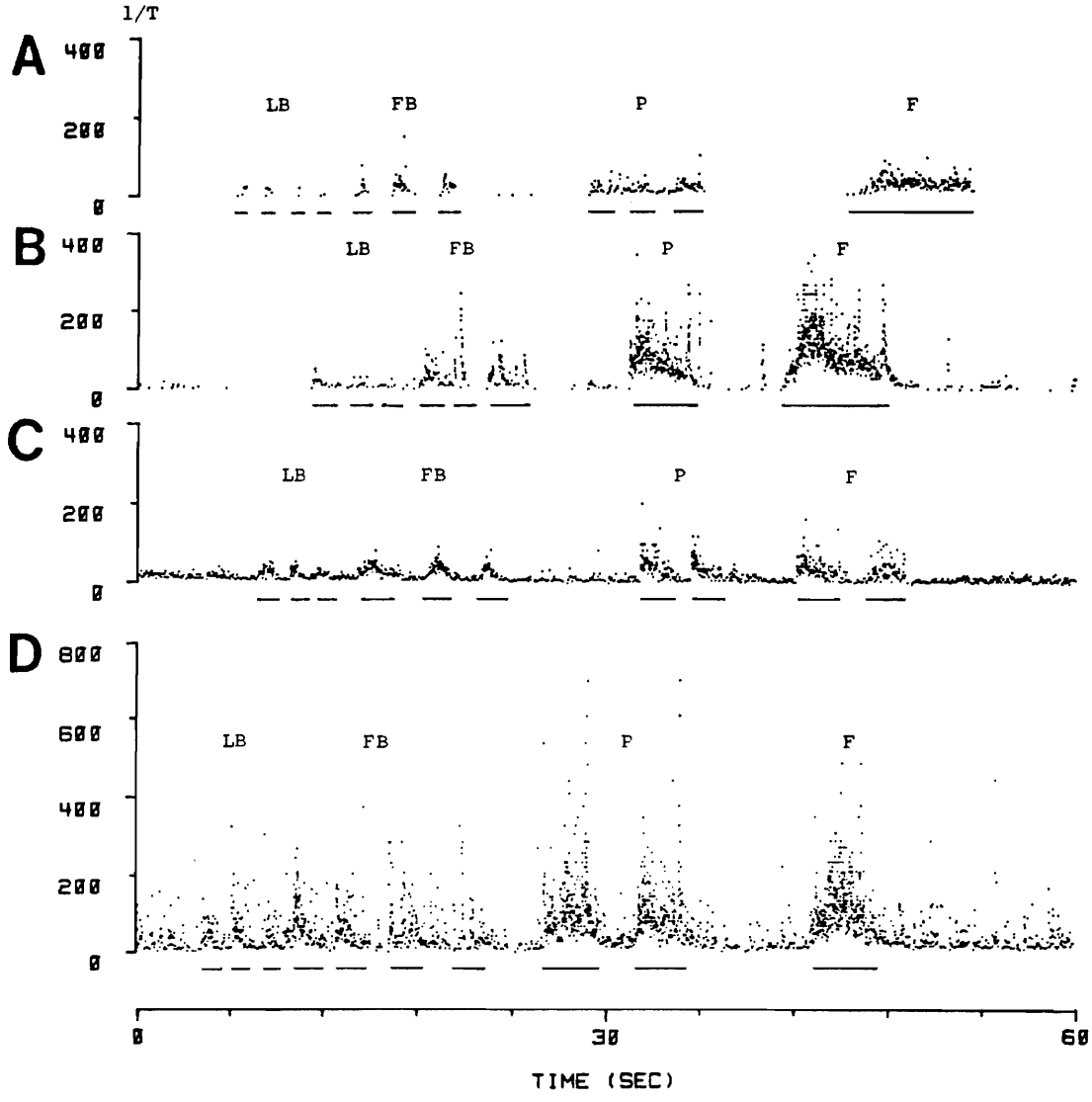
8
10
MEAN (sec)
SD
0.2
0.1
0.0
0.0
0.1
0.2
0.3
MEAN (sec)

0.001). 2) The coefficient of variation (CV=Standard Deviation / Mean X 100%) of units with intermittent resting discharge (22.09%) was greater than that of units with continuous resting discharge (7.45%).

The significance of the difference in the two coefficients of variation mentioned above was further illustrated by plotting the mean interspike interval of each unit against its standard deviation as shown in Figures 4D and 4E. Although the correlations between the mean interspike interval and its standard deviation were significant for both populations ($r=0.98$, $N=18$ for the intermittent; and, $r=0.77$, $N=29$ for the continuous resting discharge units, t test, $p< 0.001$), the slopes of their linear regression lines differed significantly (solid line for units with intermittent, slope=1.19; broken line for unit with continuous resting discharge, slope= 0.61; U test, $p< 0.01$; Yang, 1985). In summary, these differences indicate that units with intermittent resting discharge tended to have lower firing rates and greater fluctuations in discharge than units with continuous resting discharge.

Each category of ventrolateral spinal axon seemed to include units with different patterns of resting discharge. For instance, 25% of wide dynamic range units had no resting discharge (Fig. 5A), 31% had intermittent (Fig. 5B) and 44% had continuous resting discharge patterns (Fig. 5C & 5D). However, low threshold and high threshold spinal axon populations appeared to have a dominant pattern of resting dis

Figure 5. Response patterns of wide dynamic range units to mechanical stimuli. Panel A is a unit with no resting activity. B is unit with intermittent resting discharge. C shows a unit with a more regular continuous level of resting discharge than the unit in D. Note that the Y scale in panels A - C are two times than in D.


1/T
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800
600
400
200
0
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FB
P
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0
30
60
TIME (SEC)

charge. For instance, 80% of low threshold units demonstrated continuous resting discharge, whereas 62% of high threshold units had no resting discharge.

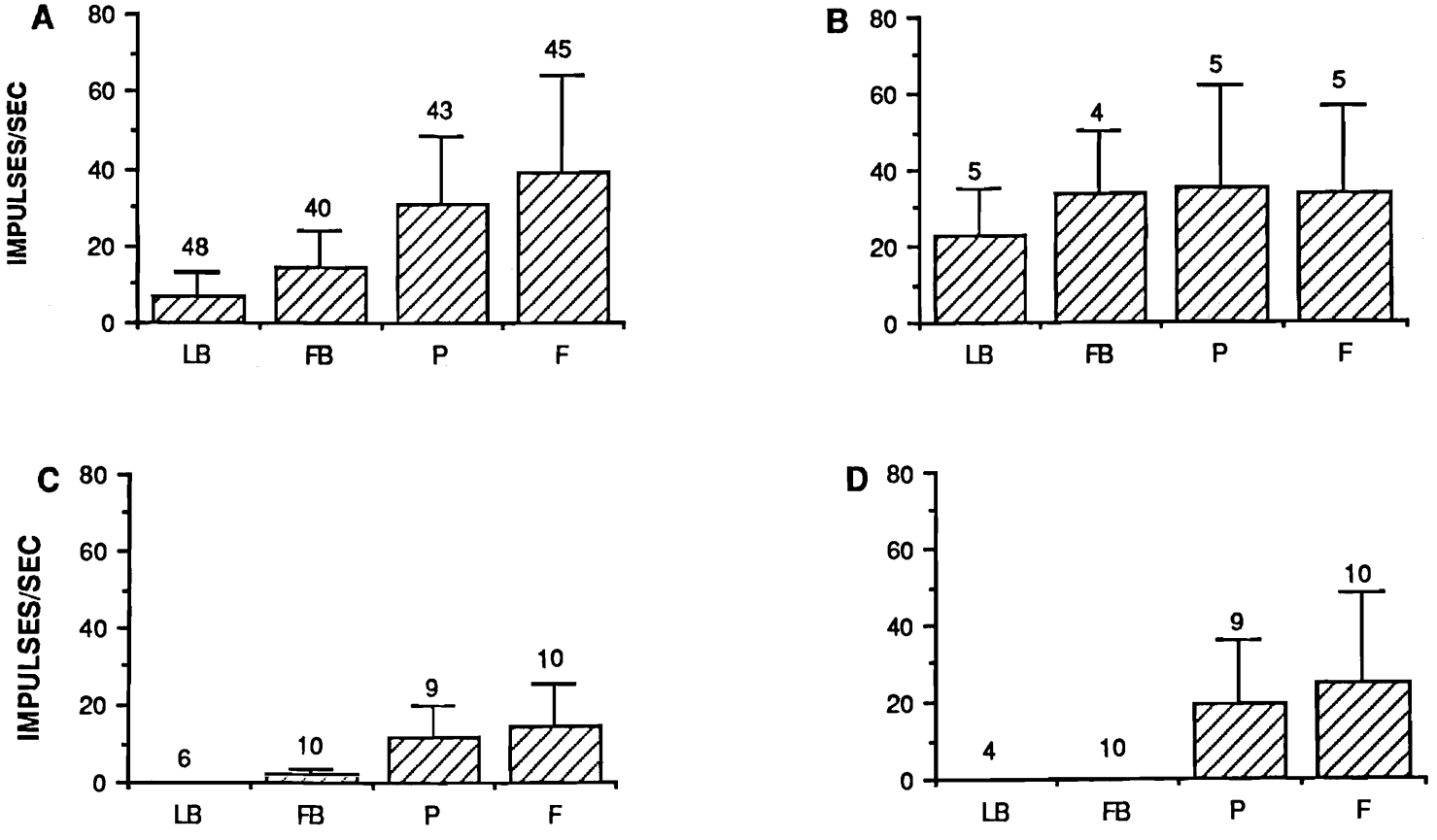
As will be presented in Part II, it is interesting to note that about 60% of pruritogen-responsive wide dynamic range units had an intermittent resting discharge pattern, suggesting that variations in resting discharge may have functional significance.

The criteria for classification

Table 1 summarizes the distribution of units in each group with respect both to their response sensitivity to mechanical stimuli and to their pattern of resting discharge. For the 92 mechanically sensitive spinal axons that were isolated from the area of ventrolateral funiculus shown in left side of Figure 1B, 57% were wide dynamic range, 23% were high threshold, and 5% were low threshold units. The remainder (15%) belonged to the "other" category. About three-fourths of the wide dynamic range and four-fifths of the low threshold units had resting activity. In contrast, almost two-thirds of the high threshold units possessed no resting discharge.

Figure 6 summarizes the response magnitude of each axon category to four grades of mechanical stimuli (LB, FB, P, and F). The resting discharge was subtracted from the response magnitude for each unit. Units from the three subtypes of each category (one subtype with no resting discharge, one with intermittent and one with continuous resting discharge)

Figure 6. Population response patterns for each axon category to four grades of mechanical stimuli. The magnitude of response is shown after subtracting resting discharge from the response magnitude for each unit. Units from the three subtypes of each category (one subtype with no resting discharge, one subtype with intermittent and one with continuous resting discharge) were combined and then the mean response magnitude was calculated. The columns represent the mean magnitudes; bars, standard deviations; the number on the top of each bar, sample size. LB: light brushing; FB: firm brushing; P: pointed probe pressing; F: forceps pinching. Panel A represents the response characteristic of wide dynamic range unit population, they responded to four grades of mechanical stimuli in a graded fashion (KW test, $p < 0.001$; MW test, $p > 0.07$ for P-vs-F, $p < 0.001$ for the other paired test). B shows the "nearly equal" response pattern of low threshold units (KW test, $p > 0.8$; MW test, $p > 0.3$ for all paired test). The response features of high threshold units are presented in panel C and D. About half of high threshold units could not be activated by LB but responded to FB, P, and F stimuli (panel C, KW test, $p < 0.01$; MW test, $p > 0.9$ for P-vs-F, $p < 0.01$ for LB-vs-FB, LB- vs-P, LB-vs-F, FB-vs-P, and FB-vs-F paired test). The other half of the high threshold units did not respond to LB and FB but could be activated by P and F mechanical stimuli (panel D, KW test, $p < 0.001$; MW test, $p > 0.7$ for LB-vs-FB and P-vs-F, $p < 0.01$ for LB-vs-P, LB-vs-F, FB-vs-P, and FB- vs-F paired test).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IMPULSES/SEC
IMPULSES/SEC
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6
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9
10
4
10
9
10
LB
FB
P
F

were combined, and then the mean response magnitude was calculated. As a population, the wide dynamic range spinal axons responded to the four grades of mechanical stimuli in a graded fashion (Fig. 6A, KW test, $p < 0.001$; MW test, $p > 0.07$ for P-vs-F, $p < 0.001$ for the other paired test). In contrast, the low threshold spinal axons responded with nearly the same response magnitudes (Fig. 6B, KW test, $p > 0.8$; MW test, $p > 0.3$ for all paired test). About half of the high threshold spinal axons did not respond to light brushing but could be activated by firm brushing and noxious mechanical stimuli (Fig. 6C, KW test, $p < 0.01$; MW test, $p > 0.9$ for P-vs-F, $p < 0.01$ for the other paired test). The other half did not respond to both either light or firm brushing. They could be excited only by noxious mechanical stimuli (Fig. 6D, KW test, $p < 0.001$; MW test, $p > 0.7$ for LB-vs-FB and P-vs-F, $p < 0.01$ for the other paired test).

## Location of axon categories

The location of different spinal axon categories in the ventrolateral funiculus was mapped in four experiments. There was no sign of population segregation (Fig. 7). This result supports the possibility that different populations of spinal axons mix together within the ventrolateral funiculus (Applebaum, et al., 1975).

## Receptive field characteristics

Table 2 summarizes the receptive field sizes of the different spinal axon categories. It shows that about one

Figure 7. Approximate locations of different axon categories in the ventrolateral white matter. Black circle, wide dynamic range spinal axon (WDR); white circle, high threshold spinal axon (HT); white square, "other" unit; cross, low threshold spinal axon (LT). Cal=1 mm.

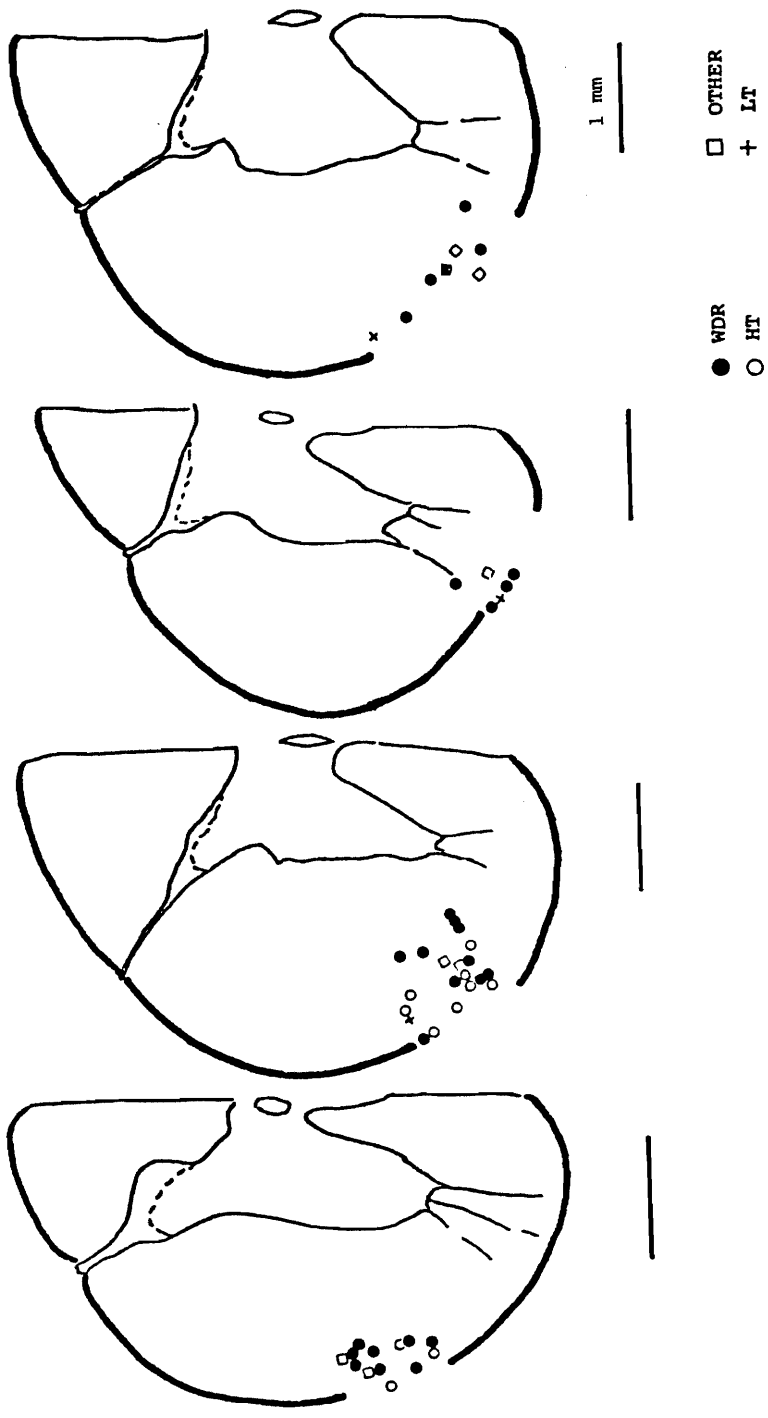

1 mm
OTHER
LT
WDR
HT

TABLE 2. Numbers of different receptive field sizes of different spinal axon categories.

| Spinal axon category | Small | Intermediate | Large | Total |
|---|---|---|---|---|
| Low threshold | 0 | 2 | 3 | 5 |
| Wide dynamic range | 8 | 31 | 13 | 52 |
| High threshold | 10 | 3 | 8 | 21 |

Explanation of headings : Small, receptive field restricted in one part of limb or lower lumbar , for instance, paw, plantar, lower leg, or part of lumbar; intermediate, restricted in one limb or lower lumbar area; large, including more than one limb or lower lumbar area. For examples see Figure 9.

half of the high threshold units had small (Fig. 8A) and the other half had intermediate to large receptive fields. The field sizes of low threshold spinal axons were intermediate (Fig. 8B) to large (Fig. 8C) and the field sizes of most wide dynamic range spinal axons were intermediate. In four experiments, the approximate locations of units with different receptive field sizes in the explored area are mapped in Figure 9. There was no obvious sign of population grouping.

Although receptive fields could be located either ipsi-, contra-, or bilaterally, each axon category seemed to have its own tendency (Table 3). Eighty percent of low threshold units had bilateral receptive fields, 22 of 52 wide dynamic range units had an ipsilateral, whereas 10 of 21 high threshold units had contralateral receptive fields. The approximate locations of units with ipsilateral, contralateral and bilateral receptive fields in the explored area were mapped and are shown in Figure 10. Again, no sign of population segregation was obvious.

**Figure 8. Examples of different receptive field sizes. Panel A shows small size (see Table 2 legend for the definitions); B, the intermediate; and C, the large size.**

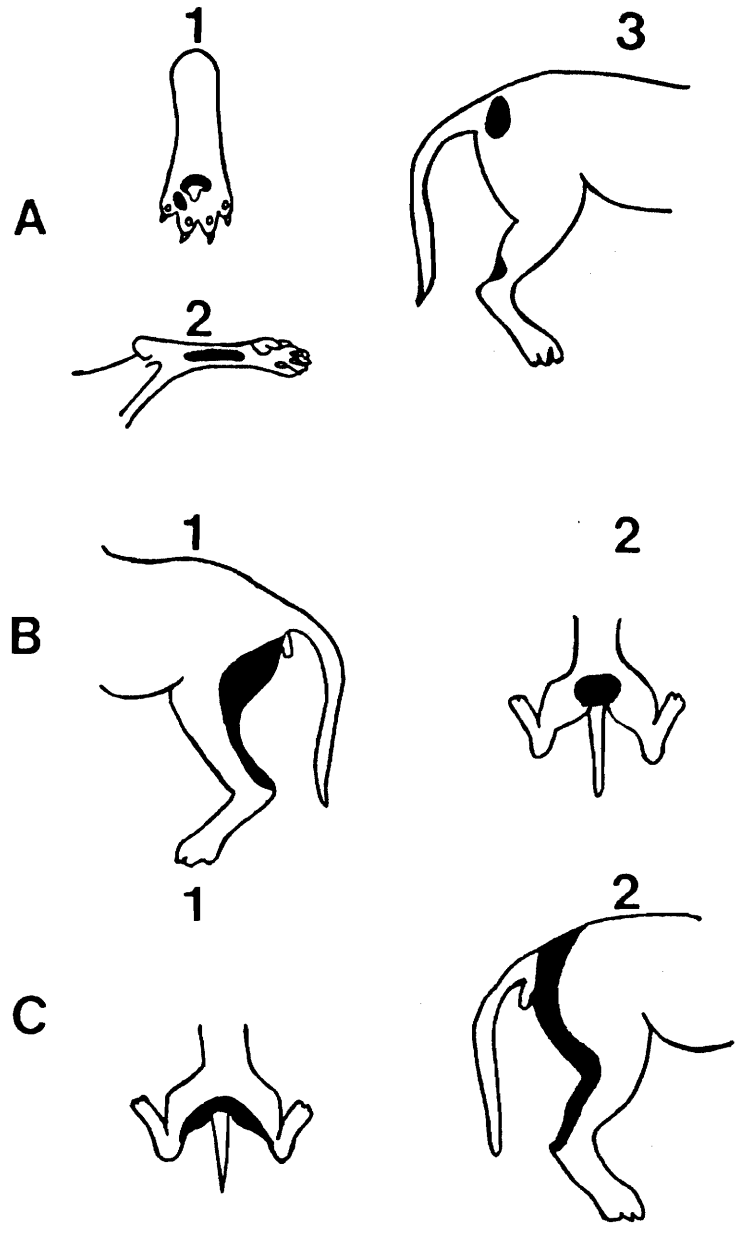

A
1
2
3
B
1
2
C
1
2

Figure 9. Distribution of units with different receptive field sizes in the ventrolateral white matter. Black circle, small size; white square, large size; star, the intermediate size. Cal=1mm. See Table 2 legend for the definitions and Figure 9 for the examples.

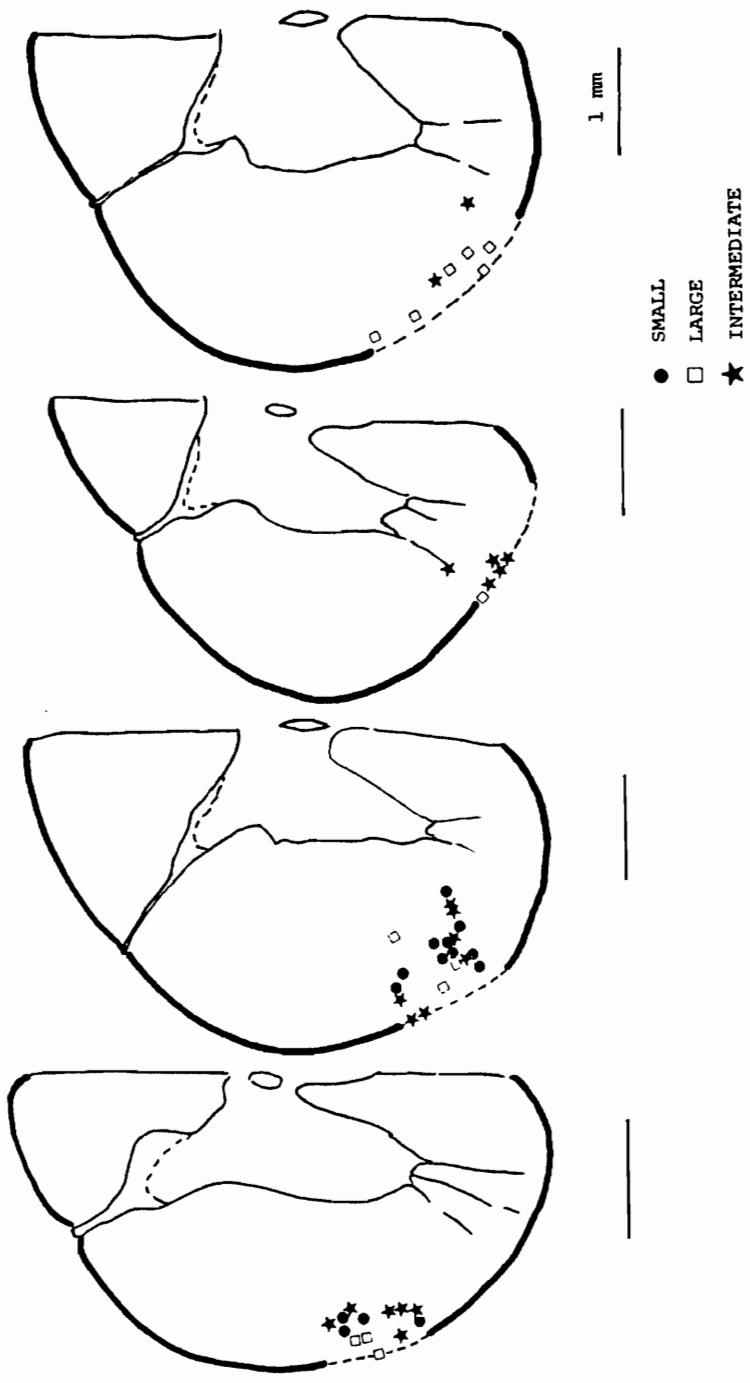
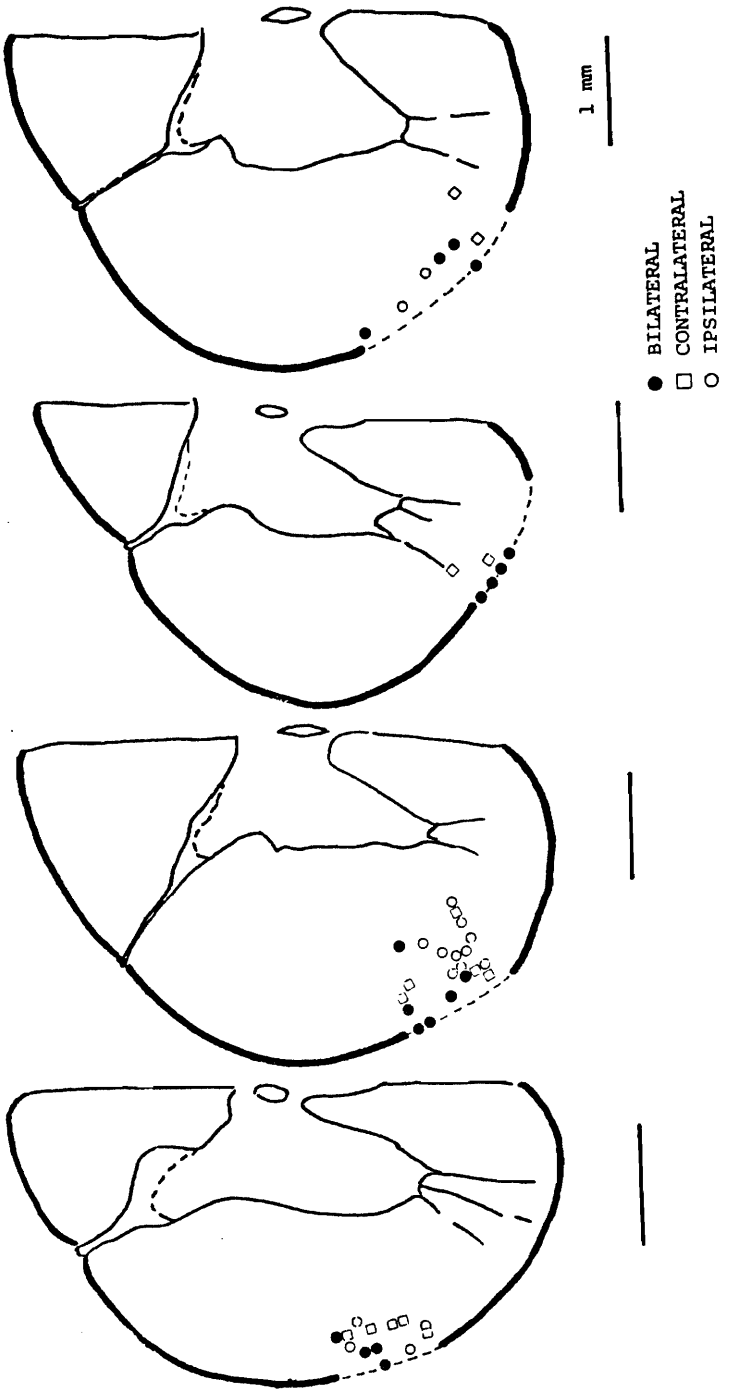

1 mm
SMALL
LARGE
INTERMEDIATE

TABLE 3. Numbers of different receptive field locations of different spinal axon categories.

| Spinal axon category | Unilateral | | Bilateral | Total |
|---|---|---|---|---|
| | Contralateral | Ipsilateral | | |
| Low threshold | 0 | 1 | 4 | 5 |
| Wide dynamic range | 17 | 22 | 13 | 52 |
| High threshold | 10 | 6 | 5 | 21 |

Figure 10. Distribution of units with different receptive field locations in the ventrolateral white matter. Black circle, bilateral; white square, contralateral; and white circle, ipsilateral receptive field. Cal=1mm.


1 mm
BILATERAL
CONTRALATERAL
IPSILATERAL

# DISCUSSION

## Significance of the present study

The classical definition of the spinothalamic tract is the spinal neurons in the gray matter with an axon which decussates and ascends in the ventrolateral white matter to the thalamus. This concept is based on clinical observations involving sensory deficits after ventrolateral cordotomies in human subjects (White & Sweet, 1969) and the studies of the anterograde degeneration in humans and animals (Willis & Coggeshall, 1978).

Based on these clinical and animal studies, attempts have been made in different laboratories to further classify spinothalamic tract neurons with the microelectrode recording technique (Willis, et al., 1974; Price, et al., 1978). Most of these investigators made their unit action potential recordings from the soma-dendritic region. The responses of these neurons to natural (orthodromic) stimuli applied to their receptive fields and to electrical (antidromic) stimulation from electrodes implanted in the thalamus were analyzed (Applebaum, et al., 1975). The advantages of this experimental protocol are that the location of the soma and the destination of the axon can be defined for individual neurons. However, the path traversed by the pro jecting axon is unknown. Therefore it has been unclear whether all these

spinothalamic tract cells had their ascending axons in the ventrolateral spinal white matter as classically defined.

Furthermore, in contrast to the wealth of data available for dorsal horn recording, comparatively few experimenters have directly recorded from ventrolateral spinal axons. Even fewer attempts have been made to classify these axons with a commonly used classification system (Fields, et al., 1970; Pomeranz, 1973). Therefore, the significance of the present study was to record directly from the cat ventrolateral spinal white matter and classify the isolated axons on the basis of the commonly used classification scheme.

The classification data in this paper served as a basis for the study of the distribution of itch-related spinal axons in the cat ventrolateral spinal white matter, and the results were compared with the numerous data from the studies of monkey spinothalamic tract cells.

## The criteria for classification

Although many classification schemes have been proposed, "...none satisfactorily represent the functions of the dorsal horn cells" (Chung, et al., 1986; also cf. Willis & Coggeshall, 1978 pp 147-153). A major cause of this disparity might be that each classification system has been developed to fulfill a different goal. For example, to investigate the afferent connection of unmyelinated (C) fibers to the dorsal horn neurons, Gregor and Zimmermann classified dorsal horn neurons in cat into four types on the basis of synaptic delay

and the response to cutaneous nerve electrical stimulation: monosynaptic cells with C response, polysynaptic cells with C response, and correspondingly, mono- and polysynaptic cells without C input (Gregor & Zimmermann, 1972).

On the other hand, for the purpose of studying pain-related tract cells, Willis and his co-workers have used different intensities of mechanical stimulation to classify spinothalamic tract neurons on the basis of their response to natural stimulation into four categories: low threshold cells, wide dynamic range cells, high threshold cells, and deep cells (Trevino, et al., 1973; Willis, et al., 1974). It is obvious that the first proposal emphasized C afferent synaptic connectivity, and therefore their classification criteria were based mainly on the electrical stimulation of peripheral nerves. In contrast, the second proposal focused on stimuli that can give rise to pain sensations, thereby having criteria for classification based on natural stimulation.

Similar differences can be found in the classification of ventrolateral spinal axons. For instance, fibers have been classified on the basis of electrical stimulation at the first of cervical spinal segment as either ascending or propriospinal spinal axons (Fields, et al., 1970). On the other hand, fibers have been grouped according to the response to different kinds of natural stimuli into a monomodal subpopulation which responded exclusively to noxious stimuli

on the skin and a multimodal subpopulation which responded to various combinations of cutaneous stimuli (Pomeranz, 1973).

It would be difficult, if not impossible, to have a system of classification criteria that would fit a wide variety of requirements. Classifying is naming. It is not necessary (or possible) that a naming scheme be able to include everything, but for effective communication it is necessary to have a basic scheme so that different people give the same name to the same objects. The most familiar example is the taxonomy of animals and plants, from which it is possible for different people to call the same species by the same name. Based on such a system further functional analysis or more detailed subpopulation clustering can be performed.

An effective way to achieve a basic naming system is to select the most obvious and important characteristics of the objects as classification criteria. These characteristics have to be clear and reproducible so that they can be recognized consistently. Based on these considerations the criteria for classifying cat ventrolateral spinal axons in this paper were similar to those that have been used to classify cutaneous receptors (Horch, et al., 1977) and pain-related spinal dorsal horn neurons (Chung, et al., 1986; Willis, et al., 1974; Dubner & Bennett, 1983), which were based on the responsiveness to different intensities of the mechanical stimuli.

The main difference in the criteria used in this paper and those used by Willis and co-workers (Chung, et al., 1986) was to incorporate the resting discharge characteristics onto the basic classification scheme. This was done for two reasons. First, different levels of resting discharge could be clearly differentiated and divided into different types. Second, evidence suggested that differences in patterns of resting discharge may have a functional significance. For example, as will be presented in PART II, about 60% of pruritogen-responsive wide dynamic range units demonstrated an intermittent resting discharge pattern.

## Ventrolateral spinal axons and spinothalamic tract cells

Because the naming system used in this classification study is the same as the system used for spinothalamic tract cells (Chung, et al., 1986), a direct comparison is possible. For instance, the percentage of low threshold, wide dynamic range, and high threshold spinal axons found in the ventrolateral white matter of cat (N=78, 6%, 67%, and 27%, respectively) closely resembles to the results obtained from monkey dorsal horn spinothalamic tract cells (see Fig. 4G to 4I of Chung et al., 1986; N=128, 13%, 65%, and 23%, respectively, chi-square test, $p > 0.3$). The percentage of the cat ventrolateral spinal axons which were activated by mechanical stimuli applied to deep tissues or by position changes of the limbs or tail (N=92, 15%) was close to that of monkey spinothalamic tract cells (Willis, et al., 1974; N=186, 11%; t

test, p >0.3). Furthermore, the intermingling distribution of different axons categories in the cat ventrolateral funiculus was similar to the monkey (Applebaum, et al., 1975).

Assuming that all axons of the monkey spinothalamic tract cells ascend via the ventrolateral funiculus, the similarities mentioned above suggest that the functional organization of cat and monkey ventrolateral funiculi are similar.

This finding differs from the common view held in recent years which has considered the distribution of pain-related spinal pathways in cat to be different from the monkey (Casey, et al., 1981; Kennrad, 1954; Willis, 1983; Willis & Coggeshall, 1978). The difference has been thought to be so obvious that it was even suggested that "the cat should be re-classified as a 'red herring'" (Willis, 1985).

It is obvious that more work should be done in this area. If the cat is a model for pain that is more similar to man than previously thought, it can be used more extensively as it has been for studies of other types of somatosensory function.

Since pain is a prototypical of somatosensory modality related to basic protective function, different animal species must have neural structures to control this function. Hence, differences as well as similarities may be anticipated. For example, in the monkey, the low threshold spinothalamic tract cells have been shown to respond much less to

pressing, pinching, and squeezing than to brushing (see Fig. 1A uppermost histogram of Chung, et al., 1986). The low threshold ventrolateral spinal axons reported in this paper responded to light and firm brushing with nearly the same magnitude as to pointed probe pressing and forceps pinching (Fig 2A & Fig. 6B). These differences may be ac counted for by differences in the animal preparations (Chung, et al., used anesthetized monkeys; in this paper, cats were spinalized). However, the differences between the percentage of low threshold spinal axons found in the cat ventrolateral funiculus (6%) and those found in monkey spinothalamic tract cells (13%) cannot be due to preparation differences. Preliminary observations on the cat dorso lateral funiculus have shown that many spinal axons dis sected from the dorsal part of the dorsolateral funiculus behave as do the low threshold of monkey spinothalamic tract cells. This finding suggests that in the cat, these axons might traverse the dorsal part of the spinal white matter.

### The distribution of different spinal axon categories in the cat ventrolateral spinal white matter

Electrophysiological studies on the monkey lateral columns have shown that the axons of spinothalamic tract axons activated by tactile stimulation intermingle with axons that respond only to high-intensity stimulation (Applebaum, et al., 1975). This observation was found to hold true for cat (Fig. 7). This kind of randomized arrangement may have

biological significance, perhaps acting as a safety factor so that injury to one part of spinal ventro lateral funiculus will not result in a complete loss of a particular sensory function.

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# PART II: THE RESPONSE OF CAT VENTROLATERAL SPINAL AXONS TO AN ITCH-PRODUCING STIMULUS (COWHAGE)

# INTRODUCTION

Itch can be defined as "an unpleasant cutaneous sensation which provokes the desire to scratch" (Rothman, 1941). If itching may be equated with scratching, it is as universal as pain (Arthur & Shelley 1959). When localized and transient, for instance from a mosquito bite, itch is a trivial nuisance; but when severe and generalized, it can become unbearable and disabling (Keele & Armstrong, 1964). For example, in patients with atopic dermatitis and the cutaneous manifestations of Hodgkin's disease, itching may lead to a vicious cycle consisting of increasingly violent scratching and itching episodes. Such scratch paroxysms may last many hours and may cease only because of somatic and psychic exhaustion of the patient (Rothman, 1941; Keele & Armstrong, 1964). There are 1.3 million Americans who suffer from atopic dermatitis (Burgess, et al., 1979; Tuckett, et al., 1984). Therefore, research involving the mechanisms of itch has not only academic but also clinical and economic significance.

It is not difficult to differentiate itch from sensations such as temperature, vibration, or from the prickling sensation which is associated with circulatory arrest of the limbs (Rothman, 1922 & 1941; Bickford, 1938). However, the sensations of itch and pain have many similarities that have

led some investigators to regard itch as a subthreshold form of pain (Bishop, 1948; Rothman 1954). For instance, both sensations can be elicited by mechanical, thermal, electrical, or chemical stimuli and are experienced as being unpleasant. Both sensations have the similar characteristics such as radiation, poor localization, poor discrimination of different intensities, and the persistence of the feeling after cessation of the stimulus (Keele & Armstrong, 1964; Rothman, 1941; Shelley & Arthur, 1957).

On the other hand, evidence has accumulated to suggest that itch and pain are indeed separate sensory modalities. For example, 1) they evoke different motor responses, scratching for itch, withdrawal for pain (Fjellner, 1981). 2) They respond differently to morphine and naloxone administration. Morphine inhibits pain but can promote itch (Hales, 1980; Bromage, 1984). Naloxone can inhibit itch in some subjects but can also lower pain threshold (Bernstein & Grinzi, 1981; Bernstein, et al., 1982; Summerfield, 1980). 3) The thresholds for eliciting itch and pain from the same area of skin are different, with a weak stimulus producing itch and strong stimulus inducing pain (Shelley & Arthur, 1957; Torebjork & Ochoa, 1981). 4) Itch and pain seem to originate from different sensory neurons which innervate different layers of the skin, itch being related to neurons that innervate more superficial layers of skin than pain fibers (Shelley and Arthur, 1957). 5) Itch and pain can be felt simultaneously in the same area of the skin and to vary

independently in intensity (Arthur & Shelley, 1959; Keele & Armstrong, 1964). 6) Gradually reducing the intensity of a pain stimulus has been reported to simply reduce and later eliminate pain without giving rise to itch (Keele, 1958; Moulton, et al., 1957). These observations strongly suggest that itch and pain are different sensory modalities and hence are subserved by different neuronal mechanisms (Burgess, et al., 1983 & 1984).

If itch is a distinct sensory modality, then according to the sensory channel hypothesis there must be an itch channel which includes the itch-related receptors, spinal cord processing circuits, spinal pathways, and the central information processing circuitry capable of producing the perception of itch (Willis & Coggeshall, 1978).

In contrast to pain and other sensory modalities, little is known of the way the itch signal is transmitted within the central nervous system. However, enough information is now available to begin investigation of this sub ject. In the peripheral nervous system, evidence suggests that the penicillate nerve endings of human hairy skin might be one of the itch-related receptors, and cutaneous unmyelinated (C) afferents might be involved in transmitting the itch signals (Cauna, 1977; Douglas & Ritchie, 1959; Tore bjork, 1974; Torebjork & Ochoa. 1981; Handwerker, et al., 1986; Tuckett & Wei, 1987b). In the central nervous system, clinical studies on the sensory loss after ventrolateral cordotomy suggest that an itch-related spinal pathway lies in the ventrolateral

spinal white matter (Arthur & Shelley, 1959; Banzet, 1927; Graf, 1960; Hyndman, et al., 1943; Taren & Kahn 1966; White,et al., 1950).

Although this literature has provided clues for where to search for itch-related spinal axons, to my knowledge no experiments have been conducted to study pruritogen-responsive spinal axons in the cat ventrolateral funiculus. The major obstacles to a successful investigation of this issue are that 1) microelectrode recording in ventrolateral funiculus is technically more difficult than from the dorsal horn soma-dendritic region, as has been mentioned in Part I. 2) The neural response to pruritus-inducing agents is not as obvious as that to mechanical or thermal stimuli. Therefore, as will be presented in this paper, the data analysis is more complicated. There is one brief report of the responses of cat dorsal horn neurons to itch-producing stimuli, but the responses were not quantified (Wall & Cronly-Dillon, 1960).

Based on this background knowledge and the classification of ventrolateral spinal axons presented in PART I, it was possible to search the pruritogen-responsive spinal axons in the cat ventrolateral funiculus using the microdis section technique described above. To my knowledge, this is the first time pruritogen-responsive spinal axons have been convincingly documented.

## MATERIALS AND METHODS

### Animal preparation

The cat was considered to be well-suited for this experimental paradigm. Because: 1) awake unrestrained cats have been shown to exhibit itch-related behavior such as scratching, twitching, and licking when the pruritogen cowhage was applied to their skin (Wall & Cronly-Dillon, 1960; Tuckett & Wei, unpublished observations); 2) anesthetized cats have been used in an extensive study of pruritogen-responsive cutaneous receptors (Tuckett & Wei, 1987 a, b); 3) their spinal axons in the ventrolateral funiculus have been classified on the basis of a commonly used classification scheme (Part I); and, 4) it has been shown that their functional organization is in some aspects similar to that of the monkey (PART I).

The units discussed in this paper come from the same population of cat ventrolateral spinal axons which have been classified into four categories as reported in PART I. Only axons associated with cutaneous afferents were used in this experiment, there belong to three categories: 1) low threshold spinal axons, 2) wide dynamic range spinal axons, and 3) high threshold spinal axons. Each category was further subdivided into three subtypes based on its pattern of resting discharge: 1) units with no resting activity, 2) units having

intermittent resting discharge, and 3) units showing a continuous resting discharge. Computer methods of data storage, display, and analysis were described previously (see PART I MATERIALS AND METHODS).

Itch-producing stimulus

The spicules of the bean plant cowhage, or Mucuna pruriens (Shelley & Arthur, 1955), have been used as a classical itch-producing stimulus since the first human psychophysical experiments on itching (Rothman, 1922). They have been applied as a clinical testing agent for evaluating the sensory deficit after anterolateral cordotomy (Hyndman & Wolkin, 1943). Cowhage consistently produced an unambiguous feeling of pruritus on human subjects (Arthur & Shelley, 1959; Broadbent, 1953; Hardy, et al., 1952, Keele & Armstrong, 1964; Rothman, 1941; Tuckett, 1982), and this effect was considered to not involve an allergic reaction (Shelley & Arthur, 1955). Hence, in the search for an effective stimulus for experimental pruritus and considering later comparisons with the existing related literature, cowhage was considered superior to other stimuli (Shelley & Arthur, 1955).

Moreover, cowhage can be inactivated by boiling (Broadbent, 1953) or autoclaving at 250$^{o}$ C for 30 minutes (Shelley & Arthur, 1955). This inactive cowhage is a useful control with which to differentiate the effect of active cowhage as will be shown in this paper. To test for inactivation,

spicules were inserted into the experimenter's skin, thus verifying a lack of pruritogenic activity.

Data from human psychophysical experiments have revealed that after cowhage insertion, although the latency, intensity and duration of pruritic sensation varied with different individuals, itching with a strong desire to scratch appeared within 15-30 seconds and lasted for approximately five or more minutes with wave-like fluctuations in magnitude (Broadbent, 1953; Hardy, et al., 1952; Keele & Armstrong, 1964, Rothman, 1941; Shelley & Arthur, 1955 and 1957; Tuckett, 1982). From this psychophysical data it was deduced that the first minute after cowhage application would be an adequate time window in which to look for the initial stages of cowhage activation of spinal pathways. Hence, in this experiment the analysis of neural response was limited to the first minute after cowhage application.

Experimental protocol

At the beginning of the experiment, the hair of both hind limbs and the lower lumbar region was clipped to about 1-2 mm. Once a unit was isolated and its receptive field perimeter defined, the remaining hair on a small portion of the most mechanically sensitive area of the receptive field was carefully removed with microscissors under microscopic magnification (12-25X). Three groups of inactive cowhage, as a control stimulus, or active cowhage, as itch-producing stimulus, were applied to an area of about 4-6 mm in diameter, each group consisting of 20-25 spicules.

Precautions were taken to minimize fatigue and sensitization (Bessou & Perl, 1969; Dubner & Bennett, 1983) of the unit under study. For instance, only mechanical stimuli were used to search for receptive fields. The intensity of the stimulation was minimized, and usually the final classification decision, as based on response to different intensities of mechanical stimulation, was made after the tests with inactive and active cowhage had been completed. Moreover, tested areas of skin were marked and not retested on subsequent units.

## Data analysis

The significance level of differences between paired data accumulated in this experiment was evaluated with the Wilcoxon matched pairs (WMP) test, which is the nonparametric analog of the parametric paired t-test. The number of nonzero values (NNZV) of the matched pairs will be specified along with the probability (p) levels in the figure legends. Unpaired data were examined by Mann-Whitney (MW) test (Siegel, 1956). Computer programs for running these statistic tests on a PC computer were available on the "Number Cruncher Statistical System" (Hintze, 1986).

## RESULTS

Once a ventrolateral spinal axon had been isolated, its response to inactive and active cowhage were recorded in paired trials. Table 4 summarizes the types and sample sizes of the paired data.

### The responses of wide dynamic range spinal axons to cowhage application

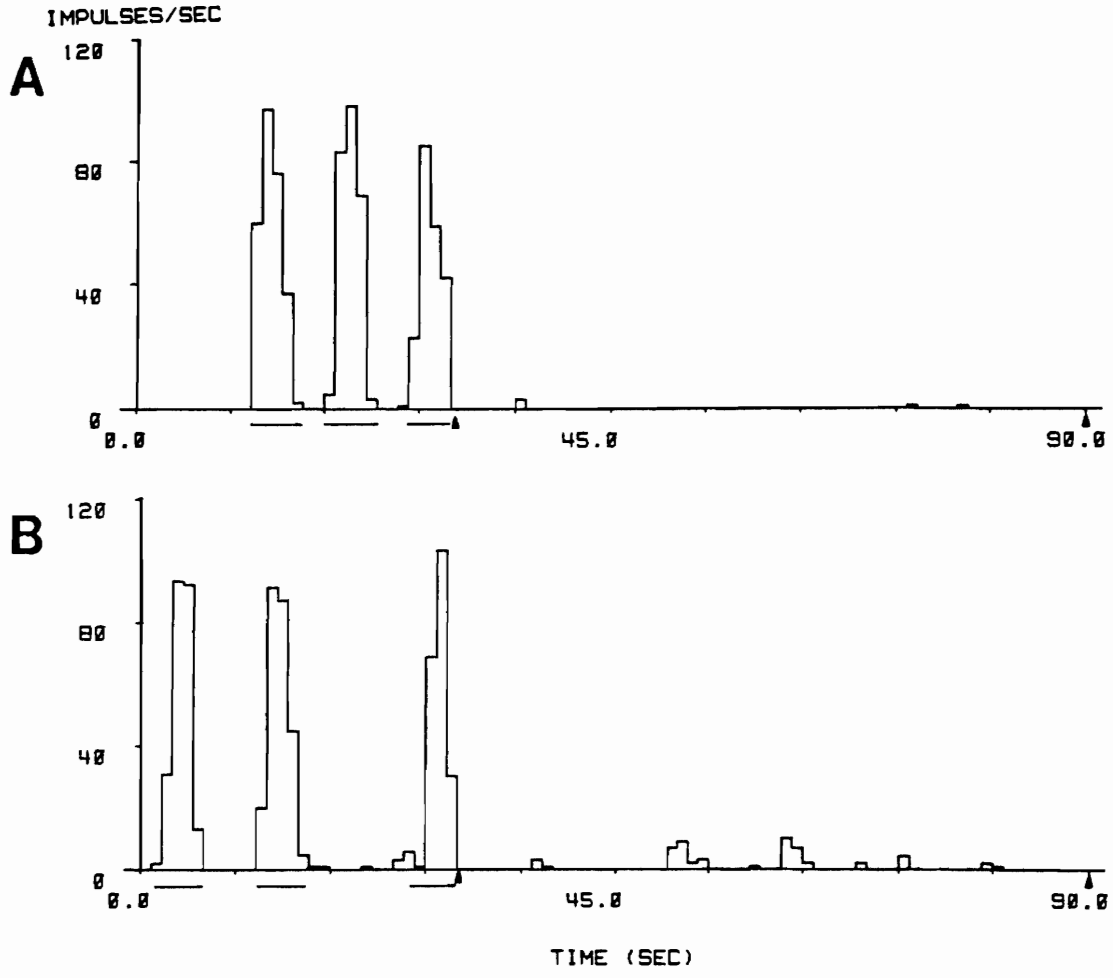
A typical inactive cowhage versus active cowhage (IC-vs-AC) paired record is shown in Figure 11. A wide dynamic range unit with no resting activity was tested with inactive cowhage (Fig. 11A) and then with active cowhage (Fig. 11B). The horizontal lines under each display mark the time of cowhage application. As mentioned in PART I, although response sensitivities varied, by definition all types of mechanically sensitive ventrolateral spinal axons could be activated by mechanical stimulation. Therefore, the mechanical pressure caused by insertion of cowhage spicules into the skin initiated a discharge which subsided after the mechanical stimulus was terminated. As shown, following active cowhage application there were bursts of activity followed by three to 15 seconds of silence that were not present after inactive cowhage application. The total amount

TABLE 4. Sample sizes from which the paired data were collected.

| Spinal axon category | RD-Vs-RD | | IC-Vs-IC | | IC-Vs-AC | |
|---|---|---|---|---|---|---|
| | No. of units | No. of data pairs | No. of units | No. of data pairs | No. of units | No. of data pairs. |
| Low threshold | - - | - - | - - | - - | 3 | 3 |
| Wide dynamic range | 11 | 25 | 10 | 11 | 34 | 47 |
| High threshold | - - | - - | 4 | 4 | 14 | 15 |

Explanation of headings: RD-Vs-RD, the resting discharge first minute spike count versus the second minute spike count; IC-Vs-IC, one minute spike count after the first inactive cowhage application versus after the second; IC-Vs-AC, one minute spike count after inactive cowhage application versus after active cowhage. These definitions apply also to the subsequent figures.

Figure 11. The response of a wide dynamic range unit with no resting activity to application of inactive (A) and active (B) cowhage. Solid lines under the impulse frequency vs time histograms indicate the time of spicule insertion. Bin width: one sec. The time interval between the two arrows was one minute. The difference in bin-to-bin discharge between the two arrows was significant (WMP test, $p < 0.01$, NNZV=17). Spikes count between the two arrows, A: 5, B: 54.


IMPULSES/SEC
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80
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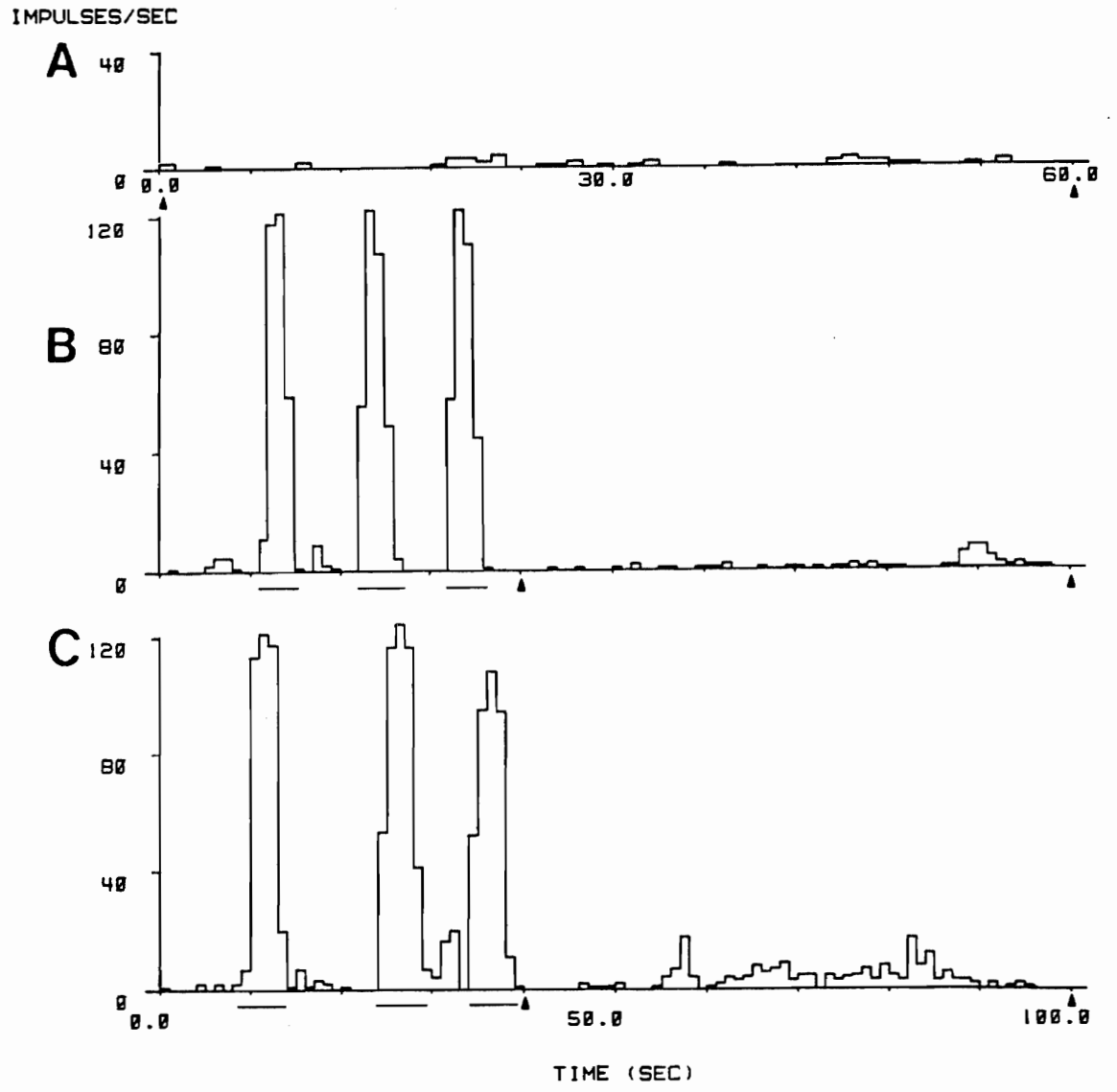
45.0
90.0
TIME (SEC)

of spikes induced by active cowhage (Fig. 11B) was significantly greater than inactive cowhage (Fig. 11A, WMP test, $p < 0.01$).

In the case of units with spontaneous resting activity, one to four minutes of resting discharge was recorded before inactive cowhage was applied (Fig. 12A & 13A). Units with intermittent and continuous resting discharge patterns are illustrated in Figures 12 and 13, respectively. For both, active cowhage typically elicited a higher rate of discharge than both inactive cowhage (Fig. 12C vs 12B, and Fig. 13C vs 13B, WMP test, $p < 0.001$ for both pairs) and the resting discharge levels (Fig. 12C vs 12A, and Fig. 13C vs 13A, WMP test, $p < 0.001$ for both pairs). In contrast, inactive cowhage sometimes induced a significantly greater number of spikes than the resting discharge level (Fig. 13B vs 13A, WMP test, $0.01 < p < 0.05$) and sometimes did not (Fig. 12B vs 12A, WMP test, $p > 0.4$).

These figures demonstrate that the response of a unit to inactive or active cowhage stimulation included two different components, the first being a response to mechanical stimulus and the second an afterdischarge. The second component represented the after effect of the mechanical stimulus in the case of inactive cowhage trials (Fig. 13B vs 13A for example), whereas, in the active cowhage trials, the after discharge was likely the combined effect of the mechanical stimulus and the pruritogenic agent found in active cowhage (Fig. 13C). Therefore, the difference between the second

Figure 12. The response of a wide dynamic range unit with intermittent resting discharge to application of inactive and active cowhage. Panel A shows impulse frequency vs time histogram of the intermittent resting discharge; B, the response to inactive cowhage; and C, to active cowhage. Bin width: one sec. Solid lines under the histograms indicate the time of spicules insertion. There was a one minute time interval between the two arrows. WMP test of the discharge between the two arrows was significant for C to B, ($p<0.001$, NNZV=47); and for C to A ($p< 0.001$, NNZV=43); but not for B to A ($p >0.4$, NNZV=37). Spikes count between the two arrows A: 41, B: 62, C: 201. Note that the Y scale in panel A is smaller then in B and C.


IMPULSES/SEC
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60.0
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B
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C
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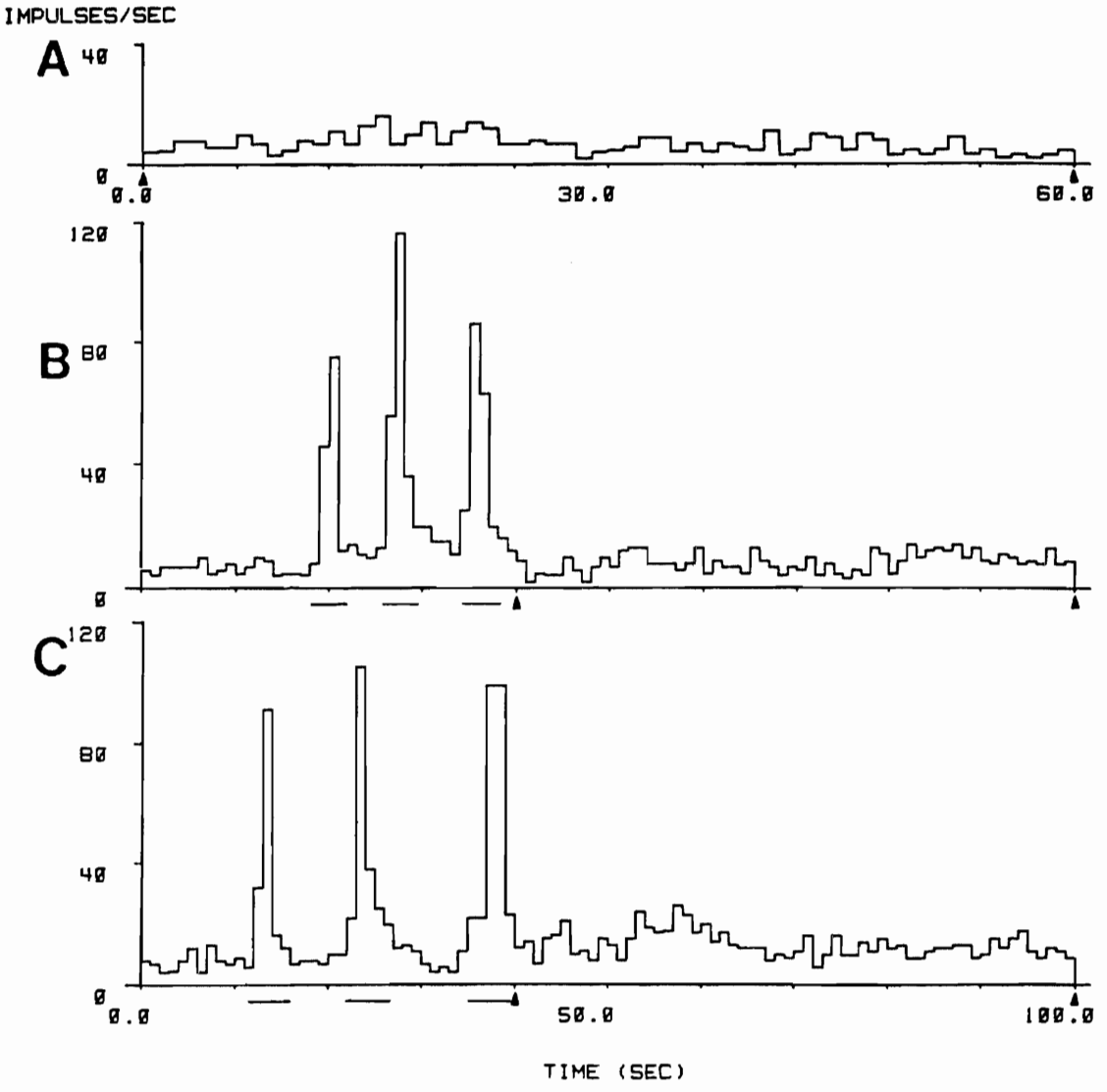
50.0
100.0
TIME (SEC)

Figure 13. The response of a wide dynamic range unit with continuous resting discharge to the application of inactive and active cowhage. Panel A shows impulse frequency vs time histogram of the continuous resting discharge; B, the response to inactive cowhage; and C, to active cowhage. Bin width: one sec. Solid lines under the histogram indicate the time of spicule insertion. There was a one minute time interval between the two arrows. WMP test between the two arrows was significant for C to B ($p < 0.001$, NNZV=56); C to A ($p < 0.001$, NNZV=59); and for B to A ($0.01 < p < 0.05$, NNZV=57). Spikes count between the two arrows A: 412, B: 503, C: 790. Note that the Y scale in panel A is smaller than in B and C.


IMPULSES/SEC
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B
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C
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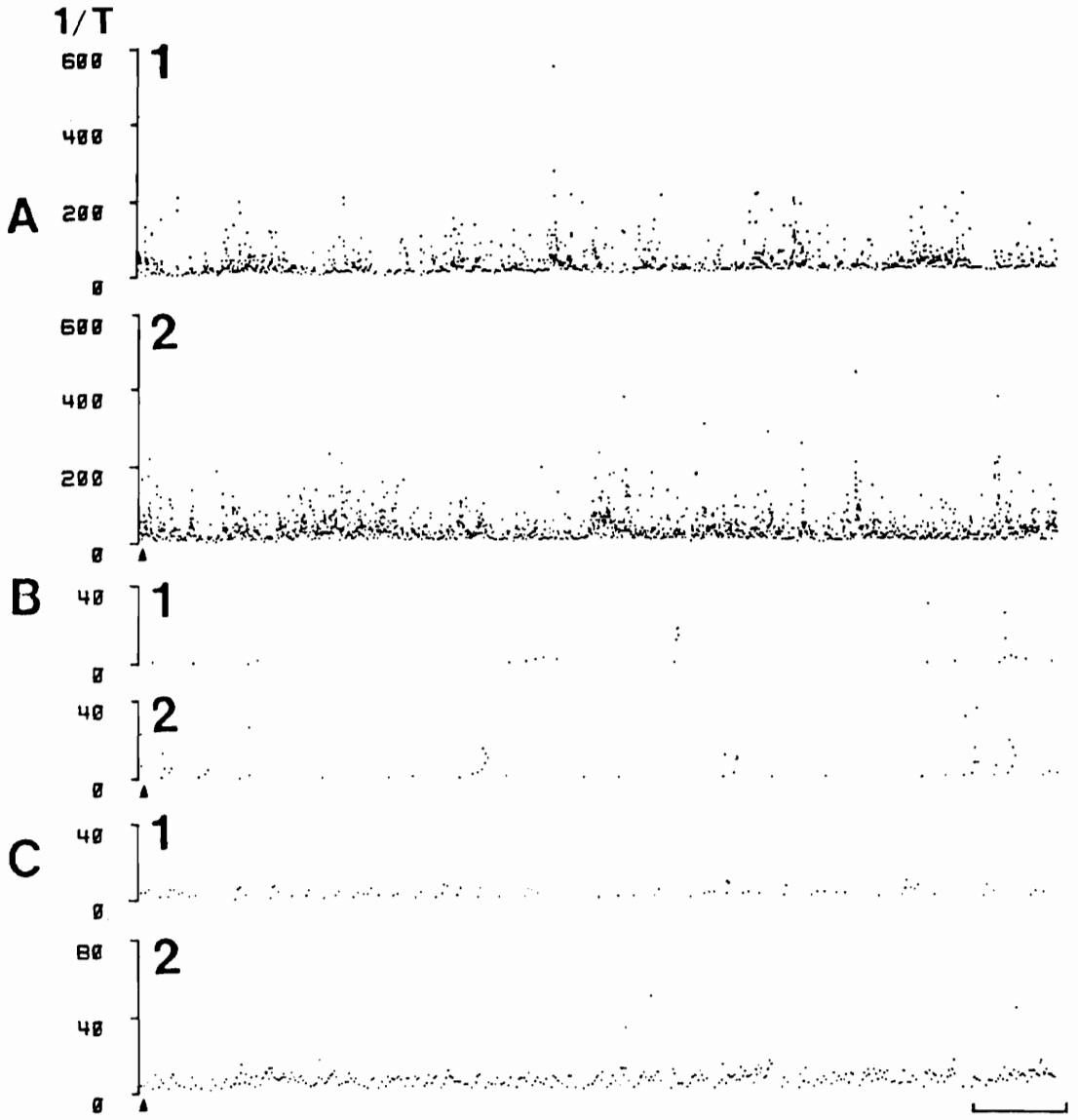
0
0.0
50.0
100.0
TIME (SEC)

components in inactive and active cowhage trials was assumed to represent the pruritogenic effect of active cowhage (Fig. 13C vs 13B).

To further investigate this issue, under microscopic observation active cowhage spicules were applied very gently into the receptive fields of three wide dynamic range units so that no direct response to the mechanical insertion was produced. After insertion these units generated a greater number of spikes (the end of the insertions were indicated by the three arrows in Fig. 14 A2, B2, and C2) than their original resting discharge level (Fig. 14 A1, B1, C1; WMP test, $p < 0.001$). This result provides additional evidence to support the notion that, aside from the response to the mechanical stimulus, there is an active agent released from active cowhage that can induce increased levels of discharge.

It is interesting to note that the pruritogenic effect of active cowhage on wide dynamic range units was not as vigorous as the direct effect of mechanical stimulation (compare the discharges underlined to the discharge between the two arrows in Fig. 11 to 13). In the case of units with resting activity (Fig. 12 & 13), the possibility existed that the difference between the second component of inactive and active cowhage trials was due to a fluctuation of resting activity with a tendency toward increasing firing rate with time. To test this possibility, it was necessary to study resting discharge versus resting discharge paired (RD-vs-RD) records. Therefore, in some experiments, resting discharge

Figure 14. Responses of three wide dynamic range units to the application of individual active cowhage spicules. Histograms of instantaneous frequency versus time showing the responses of three wide dynamic range units (A, B, and C) to application of individual active cowhage spicules. The arrow in trace 2 of each sample marks the time of the last spicule insertion. Note that there was no direct response to mechanical pressure during insertion. In each, trace 1 shows the resting discharge levels of 1076, 24, 84 impulses per minute, respectively. Trace 2 shows the afterdischarge of each unit after the insertion. The one minute spike counts are 1577, 53, and 336, respectively. The time scale is 6 second. For A1 to A2 NNZV=60 and for C1 to C2 NNZV=58 WMP test, for both $p < 0.001$; for B1 to B2, WMP test, $p < 0.01$, NNZV=30. Note that the Y-axis scaling in panel A is more than an order of magnitude greater than B and C.


1/T
600
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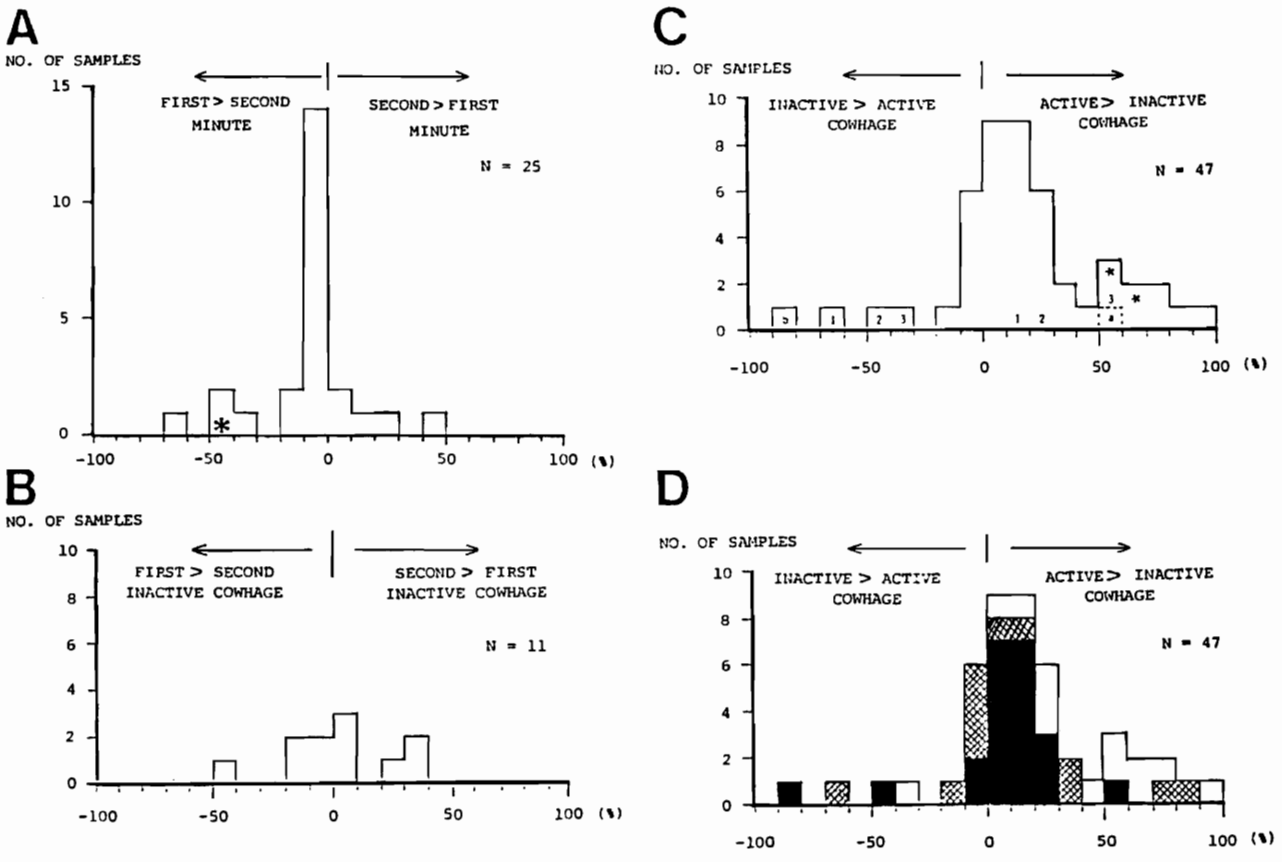
A
1
2
B
40
C
80

was recorded for two to four minutes and a comparison made between consecutive minute records.

To measure the percent difference of the firing rate during a two-minute recording period, the mean impulses per minute (IPM) in the first minute were subtracted from, and then normalized around, the mean number of impulses occur ring during the total two-minute interval. The same procedure was then followed for the mean of the second minute, with the result being the same as in the first minute, but opposite in sign. For example, a two-minute interval in which the first minute had 98 IPM and the second minute had 34 IPM would be normalized around a mean of 66 IPM and would show a "-" and a "+" 48% difference from the two-minute mean (66- 98)/66 X 100% and (66-34)/66 X 100%). The data were plotted in such a way that if the IPM during second minute was smaller than the first minute, the percentage was called negative and plotted on the left side of the histogram, and vice versa (Fig. 15). In the case cited above (the 98 and 34 IPM pair), the percent fluctuation was signed negative (-48%) and labelled with an asterisk in Figure 15A. Hence, the closer a cell was to the center of the histogram, the lesser the difference between the two values.

Figure 15A demonstrates that although the percent differences for most cells were close to the center of the histogram, more cells were distributed to the left than to the right side, suggesting a tendency for the discharge in

Figure 15. Frequency distribution histograms illustrating the response sensitivity of wide dynamic range units to the application of cowhage. A comparison was made between two values of a data pair. For instance, in panel A, the number of spikes counted in the first minute of resting discharge was compared to the second minute (RD-vs-RD). In B, the number of spikes counted in the first minute of afterdischarge after the first insertion of inactive cowhage was compared to the afterdischarge following a second insertion of inactive cowhage (IC-vs-IC). In C, a similar comparison was made between inactive and active cowhage insertion (IC-vs-AC). Sample sizes are listed in Table 4. Percent difference of paired values is used as a measure of response sensitivity (for details see the text). Percent differences on the right side of the 0% column indicate in A that the second minute spike count was greater than the first, in B that the second inactive cowhage application elicited more spikes than the first, and in C that active cowhage evoked greater activity than inactive cowhage. The converse holds true for the left side of the 0% column. Only panel C shows most of the cells being on the right side of the histogram indicating that active cowhage evoked more spikes than inactive cowhage. The large "*" in A marks of the calculation example presented in the text. The two small "*"'s and the "a" and "b", and paired numbers 1 to 3 in C represent two measurements from two receptive field spots of 5 different units. See text for discussion. Panel D is the same as C, displaying the distribution of different subtypes of wide dynamic range units. The crossed cell represents a unit with no resting discharge; the white cell, a unit with intermittent; and, the black cell, a unit with continuous resting discharge. Note the nine cells, located on the right side of the 50% column, which represents cowhage sensitive wide dynamic range spinal axons, six of which were units with an intermittent resting discharge pattern.


A
NO. OF SAMPLES
FIRST > SECOND MINUTE
SECOND > FIRST MINUTE
N = 25
B
NO. OF SAMPLES
FIRST > SECOND INACTIVE COWHAGE
SECOND > FIRST INACTIVE COWHAGE
N = 11
C
NO. OF SAMPLES
INACTIVE > ACTIVE COWHAGE
ACTIVE > INACTIVE COWHAGE
N = 47
D
NO. OF SAMPLES
INACTIVE > ACTIVE COWHAGE
ACTIVE > INACTIVE COWHAGE
N = 47
-100
-50
0
50
100 (%)

the second minute to be less than in the first. This impression was confirmed by the result of the Wilcoxon matched pairs test on these 25 RD-vs-RD pairs ($0.01 < p < 0.02$), indicating a significant tendency for firing rate to decrease with time.

Because the experimental procedure required the application of three groups of inactive, and then active, cowhage it was possible that the higher one-minute spike counts on active cowhage trials shown on Figures 11-13 were due to sensitization caused by repeated insertion of spicules. To test this possibility inactive cowhage versus inactive cowhage (IC-vs-IC) paired responses were compared. A naive situation occurred during the first experiment in this series in which the experimenter mistakenly used inactive cowhage as the active stimulus. Therefore, the data from all units obtained in this initial experiment were IC-vs-IC paired records (see Table 4 columns 3 & 4). Eleven IC-vs-IC pairs were distributed almost symmetrically with no significant shift from zero (Fig. 15B). The WMP test on 11 IC-vs-IC pairs ($p > 0.5$) also indicated that the differences were not significant.

In contrast, the IC-vs-AC pairs were distributed to the right (Fig. 15C) indicating that active cowhage induced a higher level of discharge than did inactive cowhage (WMP test on 47 IC-vs-AC pairs, $p < 0.001$). There was no tendency in either the RD-vs-RD (Fig. 15A) or the IC-vs-IC (Fig. 15B) trials for the second minute count to be greater than the

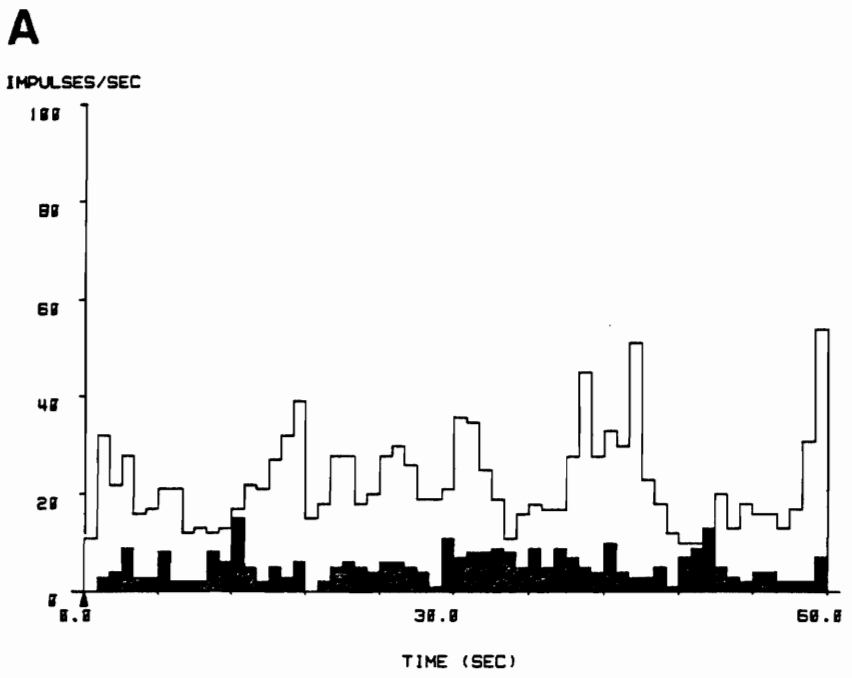
first. Hence, it appears unlikely that the active cowhage stimulation, by virtue of being second in the paired stimulus paradigm, could be the cause of the increased discharge. In fact, just the opposite was found: the active cowhage stimulus had to overcome a tendency for the second in the pair to have a lesser rate of discharge. Furthermore, a comparison of Figure 15C to Figures 15A and 15B shows that although percent differences in RD-vs-RD and IC-vs-IC pairs were less than +50%, there was a peak-count at +50% in Figure 15C.

More than 23% (eight of 34 units) of the wide dynamic range units had differences greater than +50% and may represent a subpopulation with higher sensitivity to active cowhage stimulation (for one unit, two receptive spots were tested and both had differences greater than +50%, as designated in Fig. 15C with two small "*" marks). In summary, these results suggest that the wide dynamic range population was cowhage sensitive. In addition, a subpopulation of about 23% demonstrated a relatively high sensitivity to cowhage (compare Fig. 16A vs 16B) and thus might be referred to as pruritogen-responsive spinal axons.

### Comparison of the effect of active cowhage applied to more than one spot

Ten of the 34 wide dynamic range units studied with inactive and active cowhage were tested on more than one spot within their mechanically excitable receptive fields (two spots on eight units, three spots on two units). Spots were

Figure 16. Summation of impulse frequency versus time histograms showing the population response of wide dynamic range units to inactive and active cowhage stimulation. Panel A shows the histogram of nine inactive versus active cowhage (IC-vs-AC) paired data from eight wide dynamic range units with high sensitivity to cowhage stimulation and B, the histogram of 47 IC- vs-AC paired data from 34 wide dynamic range units. Black columns represent the summation of one minute spike counts after inactive cowhage application (N=311 for A, N=19877 for B). The white columns show the summation after active cowhage stimulation (N=1336 for A, N=23435 for B). For both A and B the differences were significant (WMP test, $p < 0.001$ and NNZV=60). Note the tenfold difference in scaling of the Y-axis in A and B.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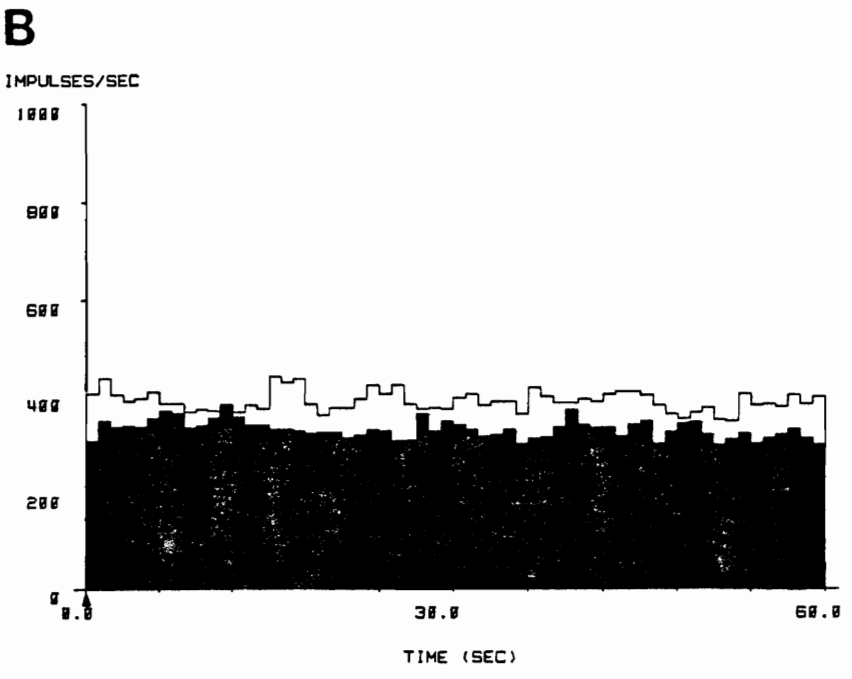
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selected on the basis of their similarity in sensitivity to mechanical stimulation. For each, the difference in response to inactive and active cowhage application was compared. Seven units had the same results at different spots on their receptive fields (an example is shown in Fig. 15C with two small "*" marks).

However, three units had a greater response to AC than IC on one spot but the opposite effect on the other spot. These effects are shown in Figure 15C with paired labels 1, 2, and 3. In other words, for three wide dynamic range units although the mechanical stimulus produced a similar excitatory effect on each tested spot, active cowhage produced more afterdischarge than inactive cowhage on one spot, but lesser on the other, raising the intriguing possibility that the cowhage defined receptive field might have inhibitory as well as excitatory areas although the whole field was mechanically excitatory.

### Effect of active cowhage applied to mechanically inhibitory receptive fields

Figure 17 shows a simultaneous recording from one spinal filament of two wide dynamic range units. Both the first (Fig. 17A top trace) and the second (bottom trace) units had resting activity. The mechanically excitatory receptive field of the first unit and the inhibitory field of the second unit overlapped, with both innervating perineum. The mechanical stimulus associated with inactive and active cowhage application (Fig. 17B & 17C solid line) excited the

first unit and inhibited the second. This effect lasted only as long as the mechanical stimulation was applied. Following inactive cowhage application, there were discharge levels similar to the resting level of activity (compare Figures 17A to 17B). In contrast, following active cowhage application, there was excitation of the first unit and inhibition of the second (compare Fig. 17C to Fig. 17A & 17B). In summary, the effects of active cowhage and mechanical stimulation were similar in that both produced an excitation of the first unit and inhibition of the second.

The second unit had a mechanical excitatory field (in addition to its inhibitory field) which was located on the thigh and separated from the inhibitory field by about five cm. When active cowhage was applied to the mechanical excitatory receptive field of the second unit a clear excitatory effect was produced (compare Fig. 18A to 18B). These two opposite effects of active cowhage on the two different receptive spots of the second unit, one within the excitatory and the other within the inhibitory receptive field of the unit, are shown in the frequency distribution histogram of Figure 15C with "a" and "b" labels.

### Response of high and low threshold spinal axons to cowhage application

Figure 19 shows the summation of impulse frequency versus time histograms of paired data from three low threshold (three pairs of data, Fig. 19A) and 14 high threshold spinal axons (15 pairs of data, Fig. 19B). The

Figure 17. Instantaneous frequency versus time histograms illustrating a simultaneous recording from two wide dynamic range units in the same filament. Panel A shows that both the first (upper trace) and second (lower trace) units had resting activity. The excitatory receptive field of the first unit and the inhibitory field of the second unit overlapped, both innervating the perineum. B the application of inactive and C of active cowhage excited the first unit and inhibited the second (the solid lines under the histograms). The afterdischarge following the inactive cowhage insertion was obviously different from the resting activity (compare A to B). In contrast, with active cowhage, the excitation of the first unit and inhibition of the second unit persisted after the active cowhage insertion (compare B and C). Time scale=10 second. There was a significant difference in activity during the time between the two arrows in the upper trace of A compared to C (NNZV=43) and B compared to C (NNZV=49) WMP test, $p < 0.001$. The lower trace of A compared to C and B compared to C were all significantly different (WMP test, $p < 0.001$, NNZV=60).

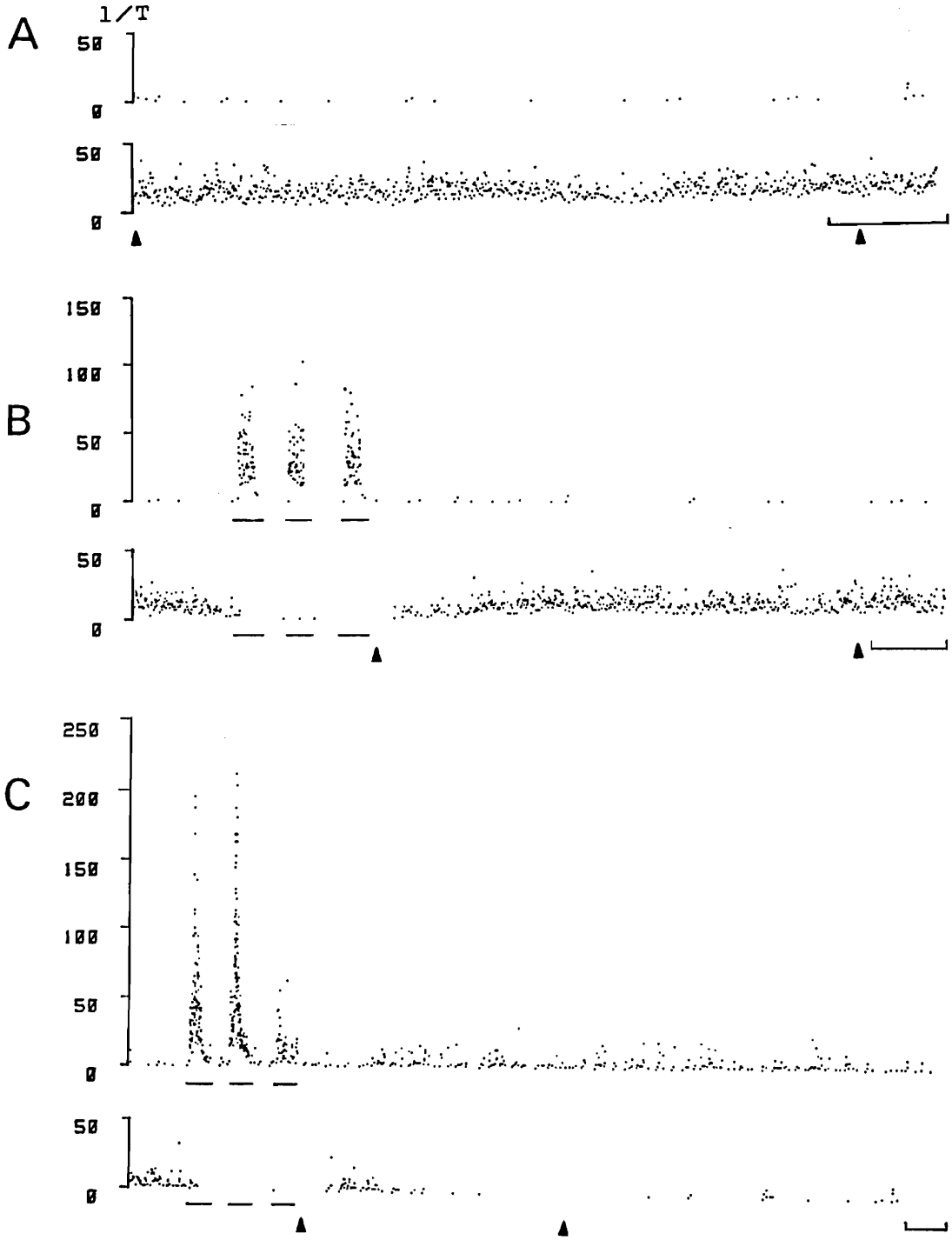
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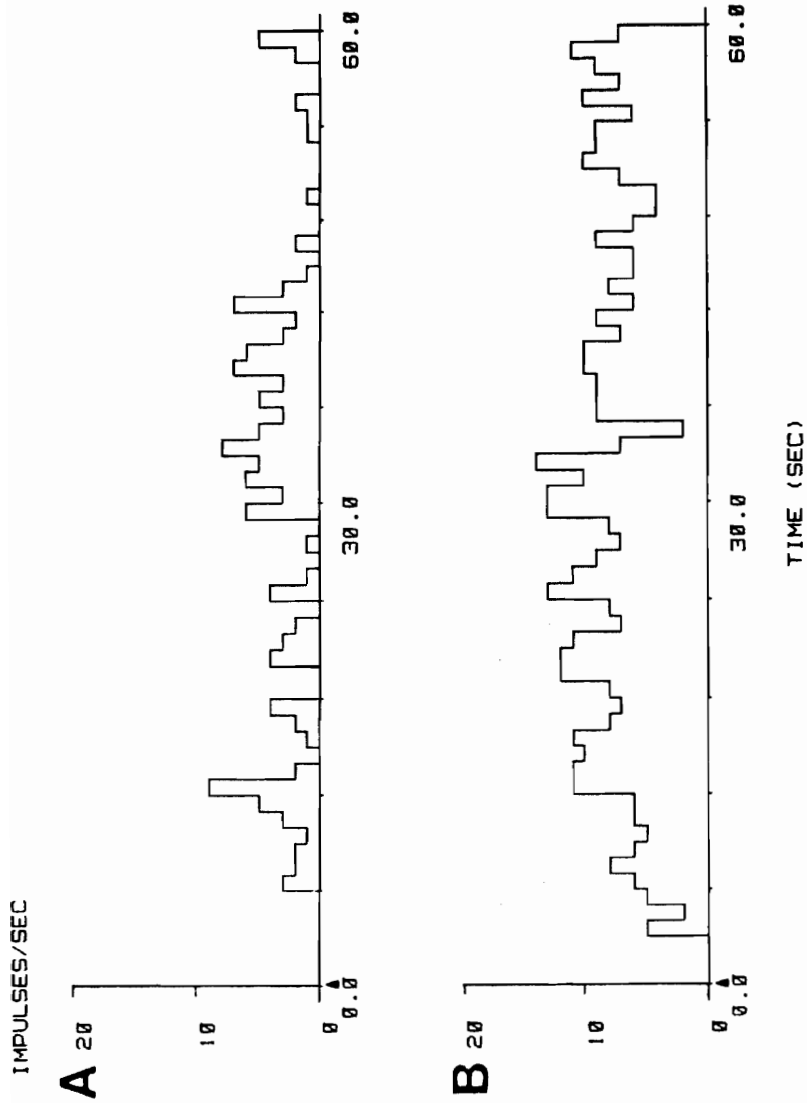
Figure 18. The response of the second unit in the Figure 17 to inactive (A) and active (B) cowhage applied on its excitatory receptive field. WMP test on A to B, $p < 0.001$, NNZV=57.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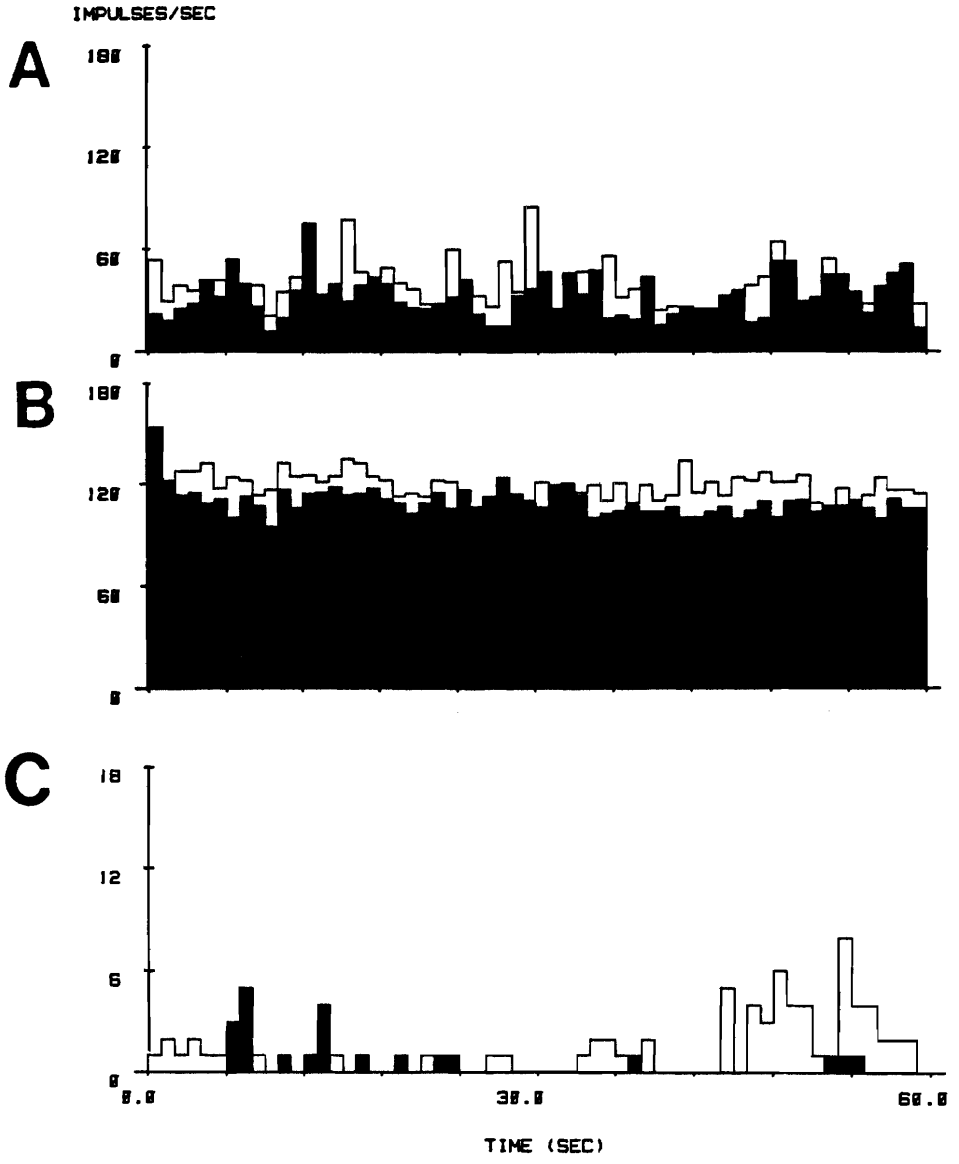
white column is the afterdischarge after inactive cowhage application, and the black column is the afterdischarge after active cowhage application. The Wilcoxon matched pairs test showed significant differences between the white and black columns (WMP test, $p< 0.01$ for Fig 19A; $p<0.001$ for Fig. 19B) indicating that inactive cowhage may have induced more afterdischarge than active cowhage. The tendency of the differences in Figures 19A and 19B (i.e., the application of inactive cowhage inducing more afterdischarge than active cowhage) was opposite to that in Figure 16, where active cowhage initiated more afterdischarge than inactive cowhage. But it was in the same direction as in Figure 15A, where the first minute resting discharge spike count was greater than the second minute. This suggests that the differences presented in Figures 19A and 19B might be due to a tendency toward decreasing firing rates with time, as occurred in the case of resting discharge (RD-vs-RD) pairs. This deduction gains additional support from the observation that the population response of four high threshold spinal axons (Fig. 19C) to an initial application of inactive cowhage was significantly greater than to a second application (WMP test, $p< 0.01$).

In summary, in contrast to wide dynamic range units, low threshold and high threshold ventrolateral spinal axons were not responsive to active cowhage stimulation.

Figure 19. Population responses of low and high threshold spinal axons to inactive and active cowhage stimulation. Summation of impulse frequency versus time histograms showing the population response of three low threshold (A, three pairs of data) and 14 high threshold (B, 15 pairs of data) spinal axons to the application of inactive (white column) and active cowhage (black column). In contrast to Figure 16 inactive cowhage induced a greater one minute spikes count than active cowhage (WMP test $p < 0.01$, NNZV=58 for A; $p < 0.001$, NNZV=60 for B). The IC-vs-IC histogram in C shows that the population response of four high threshold spinal axons to the first inactive cowhage application (white column) was also significantly greater than to a second inactive cowhage application (black column, WMP test, $p < 0.01$, NNZV=38). The spikes count in A: white column=2290, black column=1949; B: white column=7149, black column=6612; C: white column=73, black column=22. Note the Y scale in C is tenfold less than that in A and B.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# DISCUSSION

## Pruritogen-responsive ventrolateral spinal axons

A principal finding of the present study was that wide dynamic range ventrolateral spinal axons of the cat were significantly affected by the itch-producing stimulus, cowhage. Because of their location and response characteristics, these cowhage sensitive wide dynamic range ventrolateral spinal axons were speculated to be involved in relaying itch-related information within the central nervous system. However, the present study did not rule out the possibility of itch specific ventrolateral spinal axons (see APPENDIX).

To my knowledge, this is the first time an experimental model has been used to confirm the clinical concept based on observations following cordotomy that there are itch-related ventrolateral spinal axons (Banzet, 1927; Hyndman & Wolkin, 1943; White, et al., 1950; Arthur & Shelley, 1959; Graf, 1960; Taren & Kahn, 1966). This finding suggests that similarities might exist between the functional organization of the ventrolateral funiculus of human beings and cats.

Although the response was not as vigorous to cowhage as to mechanical stimulation, it was obvious. For instance, a comparison of IC-vs-IC paired data showed no significant difference between the afterdischarge following the first and

second applications of inactive cowhage. Whereas, for IC-vs-AC paired data, active cowhage produced a significantly greater afterdischarge than inactive cowhage. This effect was especially prominent in about 23% of tested units. When applied to human subjects, active cowhage initiated an unambiguous feeling of itch but inactive cowhage did not (Tuckett, 1982). Hence, the difference between active and inactive cowhage induced afterdischarge in wide dynamic range units is likely generated by the pruritogenic agent in the active cowhage and may relay itch-related information to higher levels of the central nervous system. It seems appropriate to consider wide dynamic range ventrolateral spinal axons, especially the most cowhage sensitive subpopulation, as pruritogen-responsive ventrolateral spinal axons.

## Functional heterogeneity of the wide dynamic range spinal neuron population

The term "wide dynamic range" spinal neuron was first introduced by Mendell (1966) to define spinocervical tract fibers that respond to both tactile and intense mechanical stimuli in a graded fashion.

Willis and co-workers recognized that spinothalamic tract cells are a functionally heterogeneous population and classified the cells into four categories. Their "wide dynamic range" category of the cells was behaviorally similar to Mendell's definition (Willis, et al., 1974; Chung, et al., 1986).

The potential involvement of wide dynamic range neurons in the central mechanisms of nociception has been studied extensively (Dubner & Bennett, 1983; Price & Dubner, 1977). Recently these neurons have been postulated to play a role in mediating sympathetically maintained pain (Roberts & Foglesong, 1986). Assuming the wide dynamic range spinal axons presented herein were the axons of these wide dynamic range neurons, it seems possible that wide dynamic range spinal neurons are also a functionally heterogeneous population.

In PART I, wide dynamic range spinal axons were grouped into three different subtypes according to their resting discharge pattern. Data presented in this report have shown that about 23% of wide dynamic range axons demonstrate a relatively high sensitivity to cowhage stimulation. Subdivision of these cowhage sensitive units into subtypes as shown in Figure 15D revealed that 60% of them had intermittent resting discharge. These results suggest that the differences in resting discharge pattern might correlate with the functional heterogeneity of wide dynamic range spinal neurons.

Further evidence of functional heterogeneity comes from selective stimulation of the wide dynamic range neuron population in the human ventrolateral quadrant, which has been reported to elicit different qualities of pain (i.e., burning, pricking, etc.) (Mayer, et al., 1975).

## A possible mechanism for supra-segmental extraction of sensation related to different sensory modalities

As mentioned in Part I, evidence from clinical and animal studies favors the notion that ventrolateral spinal axons may be involved in transmitting information related to different sensory modalities. According to the specificity theory of sensation, each quality of sensation is transmitted via a different sensory channel. However, data presented in this study have shown that even the lightest mechanical search stimulus used in this experiment, light brushing, could simultaneously activate the low threshold and wide dynamic range ventrolateral spinal axons (see Fig. 2A & 2B). Assuming the signals recorded from the low threshold, wide dynamic range, and high threshold ventrolateral spinal axons simultaneously ascend to suprasegmental neural circuits, it can be postulated that a coactivation mechanism might be involved in separating these signals into different qualities of sensation.

A possible coactivation mechanism can be illustrated by referring to the data in Figure 2 (Part I). First, wide dynamic range spinal axons, coactivated with low threshold spinal axons by light brushing (Fig. 2A & 2B, LB), could produce a feeling of light touch after being decoded by suprasegmental circuits. Second, in response to firm brushing, which coactivates wide dynamic range, low threshold and high threshold spinal axons (Fig. 2A, 2B, and 2C, FB), a sensation of pressure could be produced. Third, when coacti-

vation recruits high threshold axons, which only respond to overtly noxious stimuli (Fig. 2A, 2B, 2C & 2D, P & F), a sensation of pain could result. Fourth, in contrast, if only wide dynamic range axons were activated, a sensation could be initiated which would be a function of which subset of the heterogeneous of wide dynamic range population was activated. For example, according to this model, activation of pruritogen-responsive wide dynamic range axons (as shown in Fig. 11B, 12C & 13C between two arrows) would produce the sensation of itch.

This postulated mechanism is consistent with clinical observations of sensory deficits following ventrolateral cordotomy. For instance, it is thought that the reason why only minor tactile deficits can be detected after cordotomy is because of the small percentage of low threshold spinal axons in the ventrolateral funiculus (Foerster & Gagel, 1932, ref. #15 from Applebaum, et al., 1975; see also Table 1 in Part I). In contrast, because of the large percentage of high threshold and wide dynamic range axons in the ventrolateral spinal white matter, pain and itch sensations are seriously affected after cordotomy (Hyndman & Wolkin, 1943; see also Table 1 in Part I).

# APPENDIX

## Magnitude of sensation and discharge frequency

There are fundamental similarities between the neuronal activity evoked by cowhage as recorded from animal experiments and the sensory experience of human subjects following cowhage application. For example, in cats many itch-related receptors in the skin (likely, a subpopulation of the C polymodal receptor population, Tuckett & Wei, 1987b) and wide dynamic range units in ventrolateral funiculus were found to respond to active cowhage with an intermittent discharge pattern which is consistent with the wave-like fluctuations in the magnitude of itch experienced by human subjects (Shelley & Arthur, 1955; Tuckett, 1982).

However, intriguing questions remain to be answered. For instance, moderate intensities of mechanical pressure applied to the receptive fields of wide dynamic range units usually evoked a much greater discharge than did active cowhage. In contrast, the itch feeling evoked by cowhage is often as strong as or even stronger than the sensation evoked by the mechanical stimulation (Tuckett & Wei, unpublished observations). A similar divergence was also seen in the response of itch-related C polymodal receptors to mechanical and cowhage stimulation (Tuckett & Wei, 1987b).

These findings suggest that the itch-related signal must be accentuated before producing the perception of itch (cf. sensory channels hypothesis of Willis & Coggeshall, 1978).

An alternative explanation is that there may be an itch specific ventrolateral spinal axon which responds exclusively and vigorously to itch-producing stimuli but not to mechanical stimulation. Because the search stimulus used in present study was mechanical, itch-specific spinal axons might have been missed.

### The possibility of an itch specific spinal axon

Mechanical stimuli were used to search for the receptive fields of pruritogen-responsive spinal axons in the ventrolateral funiculus. When located, cowhage was consistently applied to the center of each field. Consequently, it is likely that all population of ventrolateral spinal axons that can be excited by both mechanical and pruritogenic stimuli were sampled in this study. However, if spinal axons exist that respond exclusively to itch-producing stimuli, such hypothesized itch-specific spinal axons might be underestimated or even be missed in this study.

One way to identify an hypothesized itch-specific spinal axon would be to apply an itch-producing agent over the entire body surface to be tested and then search for spinal axons with ongoing activity. There are at least two major weaknesses in this approach, both hinging on the adequacy of the search stimulus.

The first weakness would be the extreme difficulty in finding the receptive field of an activated axon. Evidence from afferent receptor and animal behavioral studies suggest that cowhage activated receptors will continue to fire for at least 30 minutes to 2 hours (Tuckett & Wei, unpublished observations). Approximate location of the receptive field would require repeated placing of a second pruritogenic stimulus on the skin and systematically searching until the discharge of ongoing activity of the recorded unit in creased. It would then to be presumed that the increase in discharge was due to activation of a portion of the receptive field.

The second weakness in this approach is that further definition of the receptive field boundary would be difficult. It would require moving the second itch stimulus and looking for further increasing activity. If none was found, it would be concluded that either the unit's response was saturated or the search had extended beyond the field boundary.

In summary, it is difficult to use a pruritogen as a search stimulus because it cannot be easily controlled; that is, it cannot be turned off as well as on.

An alternative way to search would be to record unit activity from a strand of spinal white matter which contains several spinal axons, and then use mechanical search stimuli to locate the receptive fields of all mechanosensitive spinal axons in the strand. Then active cowhage would be applied to

the center of the field, looking for recruitment of activity from units which originally were not activated by the mechanical stimulus. If recruitment were found, the cowhage-tested area of skin would be retested first with mechanical and thermal stimuli and then with more cowhage. If the spinal axon continued to display reactivity only to increasing doses of pruritogen, it would be assumed to be an itch-specific spinal axon.

An advantage of this experimental protocol would be that for each recording it would be necessary to apply only a small amount of cowhage to a restricted cutaneous area and hence the approximate field location of the itch-specific spinal axon would be immediately available.

Three conditions must be met before this protocol would become practicable. First, the receptive fields of itch specific and mechanosensitive spinal axons must have an adequate degree of overlap. Second, a somatotopic organization must exist in the ventrolateral spinal white matter. Third, mechanosensitive spinal axons must not be segregated from itch-specific spinal axons.

Condition 1: Overlapping fields. One obvious feature of pruritic sensation is poor localization (Keele & Armstrong, 1964; Rothman, 1941; Shelley & Arthur, 1957) suggesting the possibility that the itch-related channel and its associated spinal axons may have relatively large receptive fields. The data presented in Part I showed that the field size of most low threshold and wide dynamic range ventrolateral spinal

axons was intermediate to large (Table 2). Therefore, the chance of overlap between the receptive fields of itch-specific and mechanosensitive ventrolateral spinal axons would be high.

Condition 2: Somatotopic organization. Neurophysiological studies have established the existence of somatotopic organization at different levels of cat somatosensory system, including the dorsal root ganglia (Burton & McFarlane, 1973), dorsal column nuclei (Millar & Basbaun, 1975), lumbosacral dorsal horn (Brown & Fuchs, 1975), ventrobasal complex (Rose & Mountcastle, 1960), and the somatosensory cortex (Celesia, 1963). Data from the present study show that, as in the monkey's spinothalamic tract axons (ventrolateral spinal axons, Applebaum, et al., 1975), the cat's ventrolateral funiculus has a rough somatotopic organization of mechanically sensitive spinal axons (see below and Fig. 20).

Condition 3. As shown in Part I, different axon categories were mixed in the cat ventrolateral funiculus (Fig. 7) with a distribution similar to monkey (Applebaum, et al., 1975). This finding supports the concept of an intermingling distribution of different axons categories in the ventrolateral funiculus

In conclusion, the conditions necessary to search for itch-specific ventrolateral spinal axons using combined mechanical and cowhage stimuli appear to be satisfied.

The protocol used in the present experiments satisfied the above criteria, that is, during multiunit recording

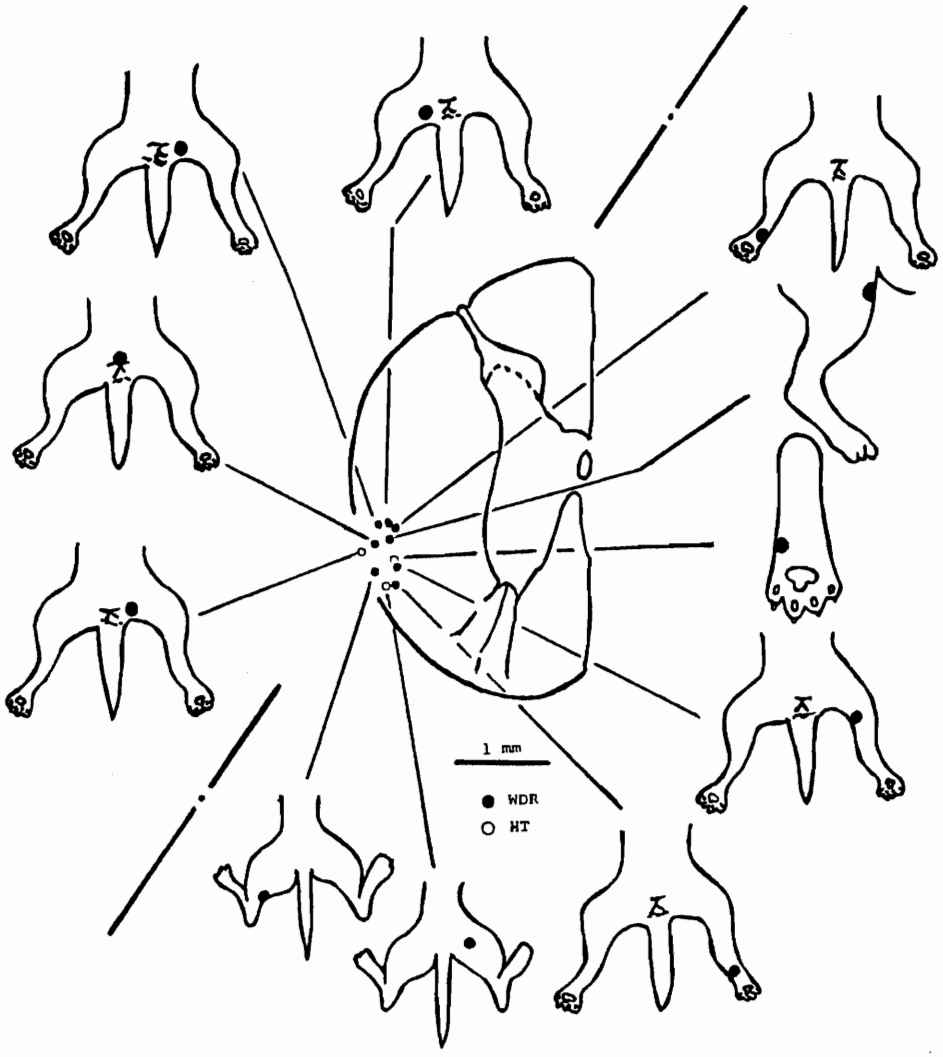
active cowhage was placed in the center of receptive fields of all mechanosensitive ventrolateral spinal axons. Since no recruitment of other axons was observed, it can be concluded either that itch-specific axons are rare in the ventrolateral funiculus, that they travel in another portion of the spinal white matter or that they do not exist at the spinal level. The present study gives no evidence to support their existence in cat ventrolateral funiculus.

### Somatotopic organization of cat mechanically sensitive ventrolateral spinal axons

In four experiments, the most sensitive portion of the cutaneous receptive field to mechanical stimulation of each unit was indicated by a black circle as shown in Figure 20. This sensitive area was used to test for responsiveness to the pruritogenic agent cowhage, as has been presented in Part II.

The location of each field was examined with reference to the placement of its corresponding axon in the ventrolateral funiculus. As illustrated in Figure 20, there appeared to be a rough somatotopic organization. Axons with fields around the perineum or base of the tail were located in the dorsolateral part of the funiculus, whereas axons with fields on the lower leg or plantar region were located in the more ventromedially. Three other experiments gave similar results suggesting that the somatotopic organization of cat mechanically sensitive ventrolateral spinal axons might be

Figure 20. Somatotopic organization of mechanically sensitive ventrolateral spinal axons. Approximate location of each axon in one experiment was plotted in the transverse plane across the spinal cord. The most sensitive portion of the receptive field is represented by a black circle. The actual receptive fields were much large than the area shown. There was a rough somatotopic organization showing that the caudal parts of the body were represented in the dorsolateral portion of the ventrolateral funiculus, whereas the more rostral parts of the body were represented more ventromedially. WDR: wide dynamic range spinal axon. HT: high threshold spinal axon.


1 mm
WDR
HT

similar to monkey spinothalamic tract (compare Fig. 11 of this paper to Fig. 12 of Applebaum, et al., 1975).

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## CONCLUSION

1) In the cat, spinal axons in the ventrolateral funiculus have been classified into low threshold, wide dynamic range, high threshold, and "other" categories on the basis of a commonly used classification scheme. The results based on this classification can be directly compared to the existing related literature, especially to data obtained from monkey.

2) Assuming that all axons of monkey spinothalamic tract cells reported by Willis's research group ascend to thalamus via the ventrolateral funiculus, the results presented in Part I suggest that the functional organization of the cat ventrolateral funiculus is similar to monkey in terms of a) the percentage of each axon category, b) the intermingling of each axon category in the ventrolateral funiculus and c) the somatotopic organization of the mechanically sensitive spinal axons in the ventrolateral spinal white matter.

3) Although there is a chance that hypothesized itch specific spinal axons might have been missed by the mechanical search stimuli used in present study, evidence is given in Part II that wide dynamic range ventrolateral spinal axons of cats were significantly affected by the classical itch-producing agent, cowhage. This is the first time an animal model has been used to confirm the clinical concept, gained from observation following cordotomy, that there are

itch-related axons in the ventrolateral funiculus. This finding suggests that the functional organization of the ventrolateral spinal white matter of human beings may have similarities to that of cats.

4) Patterns of resting discharge of spinal axons have been defined and incorporated into the commonly used classi fication scheme, so that each axon category could be further subgrouped into three subtypes. About 60% of cowhage sensitive wide dynamic range ventrolateral spinal axons were found to have an intermittent pattern of resting discharge. This result suggests that the different patterns of resting discharge may have functional significance.

# C U R R I C U L U M  V I T A E

Name: Jen Yu Wei
Birthdate: January 26, 1938
Place of birth: Fukien, China
Nationality: American citizen
Sex: Male

## Education

Fu-Dan Univ., Shanghai, China 1957-1962
(Certificate of Graduation in Specialty of Human & Animal Physiology, Department of Biology, 1962)
Shanghai Institute of Physiology 1962-1966
Academia Sinica, Shanghai, China
(Certificate of Graduation [Ph.D. equivalent] in Neurophysiology, 1966)
University of Utah 1987
Ph.D. in Sensory Physiology

## Grants

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