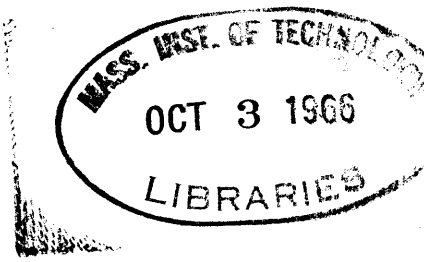

PYRAMIDAL TRACT EFFECTS ON SPINAL CORD INTERNEURONS

by

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ABSTRACT

Pyramical tract effects on spinal cord interneurons

By Eberhard E. Fetz

Submitted to the Department of Physics on August 22, 1966 in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The effects of stimulating the pyramidal tract (PT) on interneurons in the dorsal horn of the cat's lumbar spinal cord was investigated. These interneurons were characterized according to the laminar organization found anatomically by Rexed and physiologically by Wall.

Unilateral stimulation of the medullary pyramids with trains of square current pulses evoked the following types of responses: (a) flexion of the contralateral hind limb, (b) field potentials in the lumbar spinal cord, (c) dorsal root potentials in contralateral dorsal rootlets, and (d) changes in activity of single cells.

The field potentials were analyzed in a preliminary study to locate the cells excited by the PT. The source density of the field potentials, as defined by their Laplacian, was found to be greatest in layers 5 and 6.

The negative dorsal root potentials (DRP's) evoked by PT stimulation were shown to correlate with a decreased effectiveness of afferent volleys in evoking responses in certain dorsal horn cells and in evoking a DRP. A late positive phase of the PT-DRP was observed, but shown not to correlate with hyperpolarization of the dorsal root fibers.

Single cells were characterized in terms of layers on the basis of their histologically verified depth and their responses to natural stimulation of the skin and movement of joints. The possibility of axonal projection into the dorsolateral column (DLC) was tested by antidromic stimulation. The PT was found to inhibit about half the layer 4 cells and leave half unaffected. Layer 5 cells were equally divided among unaffected, inhibited, excited, and showing mixed inhibition and excitation. Three-fourths of the layer 6 cells were excited. Of the layer 4 and 5 cells with axons in the DLC, 80% exhibited pure or mixed inhibition, indicating a preferential inhibition by the PT of cells with ascending axons.

These results, combined with other findings, support the hypothesis that the inhibition seen in layers 4 and 5 is at least partly presynaptic and mediated by PT fibers from sensory cortex. The excitation in layers 6 and 5 may be largely direct and evoked by PT fibers from motor cortex.

Thesis Supervisor: Patrick D. Wall

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BIOGRAPHICAL NOTE

Eberhard Fetz attended Rensselaer Polytechnic Institute from 1957 to 1961 as a physics major. At the end of his freshman year he received the "Beginning Physics Achievement Award", and in 1961 was elected associate member of Sigma Xi. With a 97% grade average he graduated cum laude and first in his department.

In 1961 he began graduate work in physics at M.I.T. Becoming interested in the biophysics of the nervous system, he worked with Dr. George L. Gerstein on a model simulating the spontaneous activity of auditory neurons, the results of which were summarized in a joint paper: "An RC Model for Spontaneous Activity of Single Neurons" by E.E. Fetz and G. L. Gerstein, M.I.T. Research Laboratory of Electronics, Quarterly Progress Report #71, October 15, 1963, pp. 249-257.

In 1964 he began research on the frog spinal cord with Dr. Karl Kornacker and Prof. P.D. Wall, in an attempt to determine the location of endings of various afferent nerve groups by source-sink mapping of synaptic potentials. In 1965 he began the present investigation of pyramidal tract effects on spinal cord interneurons in the cat, under the direction of Prof. P.D. Wall.

He was elected full member of Sigma Xi in May, 1966.

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INTRODUCTION

Two of the major functions of spinal cord interneurons are integrating local reflexes and transmitting information from the skin and muscles to higher centers. These functions are in turn subject to control by higher centers via impulses in descending pathways. One of these pathways, the pyramidal tract (PT), forms a direct connection between cells of the cerebral cortex to those of the spinal cord. Originally this tract was thought to function only as a "motor" pathway, relaying orders concerning the execution of movements. More recently this tract has been found capable of exerting substantial control on the transmission of afferent impulses. Thus, the PT influences both functions of the spinal cord cells: the formation of reflexes and the relaying of information in ascending tracts.

To unravel the dual function of the PT one would have to investigate its effects on various types of spinal cord cells. In the cat almost all the neurons contacted by the PT are found in the dorsal horn of the gray matter. These cells have recently been characterized physiologically as well as anatomically, so an investigation of PT effects on these dorsal horn interneurons is of considerable interest.

I. Some Relevant Studies of PT Function.

The PT consists of the axons of cells in the cerebral cortex which travel via the medullary pyramids into the spinal cord. Only in the bulbar pyramids is this tract relatively uncontaminated by other fiber systems. The PT is found only in mammals, its size increasing along the phylogenetic scale; cats have around 186,000 PT fibers, man about 1,200,000 (Lassek, 1954). The best known cells of origin are the large Betz cells in area 4 of the cortex, but these contribute only the largest fibers, about 2% of the total. Estimates vary concerning the relative contribution of different cortical areas. Ruch (1960) concludes that in man the precentral "motor" cortex (areas 4 and 6) contributes 40% of the PT axons; the postcentral "somatosensory" cortex (areas 3-1-2) contributes 20%, while the rest of the cortex supplies the remaining 40%.

A unilateral section of the PT produces movement disorders in the contralateral limbs, of varying severity in different species. After severing the PT of cats, Tower (1935) observed a loss of phasic activity, especially flexor, with no loss of postural, or tonic, activity. Specific reactions, like placing, pawing and clawing were diminished or lost, and threshold of "spinal flexor centers" was increased. She observed that although noxious stimuli on the limbs evoked flexion at higher thresholds after PT section,

they evoked blinking at the same threshold. She concluded that the motor, but not the sensory system was affected. Liddell and Phillips (1944) made similar observations, but also noted an increased tone in extensor muscles.

Stimulating the PT generally evokes flexion of the contralateral limbs. Landau (1952) stimulated the medullary PT of decerebrate cats and recorded the EMG of various hindlimb muscles, as well as observing gross responses. Although flexion was the predominant response, movements were found to be quite variable from one cat to the next, and from time to time in the same animal. After carefully controlling all experimental factors, he attributed the variability of responses to the spinal mechanisms upon which the PT acts. The EMG showed that muscle movements were evoked and suppressed by the PT, but synergists and antagonists were not always reciprocally affected.

Lloyd (1941) was the first to examine the effects of PT stimulation on spinal cord interneurons. He introduced the technique, also adopted here, of sectioning the medulla except for the PT and stimulating rostral to this cut (see METHODS). With a single shock to the medullary pyramid, the mass discharge recorded in the PT at lumbar levels began about 4.5 ms. later and continued for 10 or more ms. Thus, the fastest fibers were estimated to conduct at about 65 m./sec. Lloyd located the earliest responding interneurons in the "external basilar region" -- i.e., the lateral portion of Rexed's laminae 5 and 6 (Fig. 1). With additional

PT shocks, cells in the "internal gray nucleus of Cajal" -- i.e., the medial region of laminae 6 and 7 -- were reported to be excited. Testing the monosynaptic ventral root reflex after a conditioning PT train, Lloyd demonstrated a facilitation of the reflex beginning 12 - 20 ms. after the start of PT stimulation. The disynaptic reflex ("3-neurone reflex arc") was also facilitated, but at even shorter latencies. Lloyd concluded that the PT effects were mediated through the interneurons in the base of the dorsal horn.

Hagbarth and Kerr (1954) were the first to demonstrate that higher centers could influence the conduction of afferent impulses in ascending spinal tracts. They showed that the afferent volley in the ventral columns could be depressed by stimulating various central structures, including the pre- and postcentral cortex, somatic sensory area II, and reticular formation. In addition the evoked responses in the midbrain, cerebellar and sensory cortex were observed to be depressed by such stimulation. Anesthesia with pentobarbital or chloralose completely abolished the inhibitory effect of central stimulation. Recording from single axons ascending in the dorsolateral column Hagbarth and Fex (1959) found cells excited and inhibited by stimulation of cerebral or cerebellar cortex.

In recent experiments Eccles and Lundberg and co-workers have classified afferent nerves and interneurons according to their responses to electrical stimulation. Character-

izing various muscle and cutaneous afferent nerves according to their origin and threshold to electrical stimulation, and characterizing spinal cord neurons according to their input from various sources, these workers are attempting to unravel the functional interconnections of these elements.

The effects of stimulating sensory-motor cortex on lumbo-sacral interneurons was investigated by Lundberg, Norrsell and Voorhoeve (1962), who recorded intracellularly and stimulated peripheral nerves electrically. They found cortically evoked excitatory post-synaptic potentials in interneurons receiving excitation from "flexor reflex afferents" (cutaneous afferents, group II and III muscle afferents and high threshold joint afferents), and group I muscle afferents. Some interneurons receiving exclusively cutaneous input also received excitation from cortex, but these did not give rise to ascending axons; those with ascending axons were not excited by cortical stimulation. No information was given on the location of these interneurons.

In a similar study Lundberg and Voorhoeve (1962) investigated the cortical effects on spinal reflex arcs by intracellular recording from motoneurons. A cortical facilitation of polysynaptic pathways mediating various reciprocal effects was found. Cortical stimulation could also evoke synaptic effects on motoneurons, presumably via interneurons; excitatory action predominated in flexor motoneurons, and inhibitory effects in extensors, although mixed effects were also seen.

Carpenter, Lundberg and Norrsell (1963) demonstrated that descending inhibitory effects from the cortex may be presynaptic. They stimulated sensory-motor cortex of lightly anesthetized cats and observed dorsal root potentials in lumbar dorsal roots. By recording inside afferent fibers and testing their excitability (Wall, 1958), they demonstrated a cortically evoked depolarization of cutaneous and group Ib, II and III muscle afferents.

Lundberg, Norsell and Voorhoeve (1963) investigated the effects of stimulating sensory-motor cortex on various ascending spinal pathways. Most of these pathways were influenced the same way from cortex and flexor reflex afferents; the only exception was the spinocervical tract, which was activated by FRA's but not cortex. The only cortical effect reported on this tract was an occasional weak inhibition of response to peripheral stimulation, which was attributed to presynaptic inhibition.

In a systematic investigation of properties of cells of the spinocervical tract Taub (1963) demonstrated descending inhibition from numerous regions of the bulbar and pontine reticular formation and the cerebellar nuelei. Although the PT was presumably also stimulated at some of the pontine loci, no specific stimulation of this tract was attempted.

Andersen, Eccles and Sears (1963) demonstrated that the region of the sensory-motor cortex from which dorsal root potentials could be evoked was somatotopically organized. In

addition, they showed that the precruciate cortex evoked DRP's not directly, but via projections to the postcruciate cortex. Recording intracellularly from spinal cord interneurons, they verified that some were excited by cortical stimulation, while others exhibited inhibition presumed to be presynaptic, since it parallelled the depolarization of the afferent fibers. They made "no systematic study... of cortical inhibitory action", so they apparently did not look for inhibitory post-synaptic potentials. Their methods precluded characterizing these cells according to their response to natural stimulation or the location of their cell bodies.

The first systematic characterization of dorsal horn interneurons by their location and response to natural stimulation was attempted by Wall (1966), who also investigated descending influences from cortex and brain stem on these cells. The cells distinguishable on the basis of their physiological responses were found to correlate with groups distinct on the basis of anatomical criteria. A brief review of the anatomical structure of this region is necessary before discussing Wall's physiological findings.

II. Anatomical organization of the dorsal horn.

Rexed (1952, 1954) divided the spinal cord gray matter into different regions, called laminae, on the basis of the anatomical characteristics of various cell groups. As shown in Fig. 1, the laminae described by Rexed in the dorsal horn

forms approximately horizontal layers.

Lamina 1 is a thin marginal layer covering the top of the dorsal horn and bending around its lateral side. It contains loosely distributed small, medium and large cells, often spindle shaped, with their long axis parallel to the lamina surface. Their cell bodies range from 5 x 8 μ to 15 x 30 μ. Szentagothai (1964b) observed that the dendritic arborization of these cells generally remains in layer 1, although dendritic branches of some cells turn ventrally into layers into layers 2 and 3. He traced the axons of these cells to Lissauer's tract.

Lamina 2 contains tightly packed, small cells, with diameters between 5 and 10 μ; these are either rounded or spindle shaped, with their long axis perpendicular to the layer surface. These cells exhibit little cytoplasm and have rich dendritic arborizations origination from one or two poles of the cell body, confined to layers 2 and 3 and oriented perpendicular to their surface.

Lamina 3 cells resemble those of lamina 2 in shape and orientation, but differ in being slightly larger (average 7 x 11 μ) and more varying in size and less closely packed. Although Rexed identified the classical "substantia gelatinosa" with lamina 2 only, some investigators (Szentagothai, 1964b, Wall, 1965, Sprague and Ha, 1964) include lamina 3 as well, because of its similar structure. However, Ralston (1965) has recently found additional distinctions between

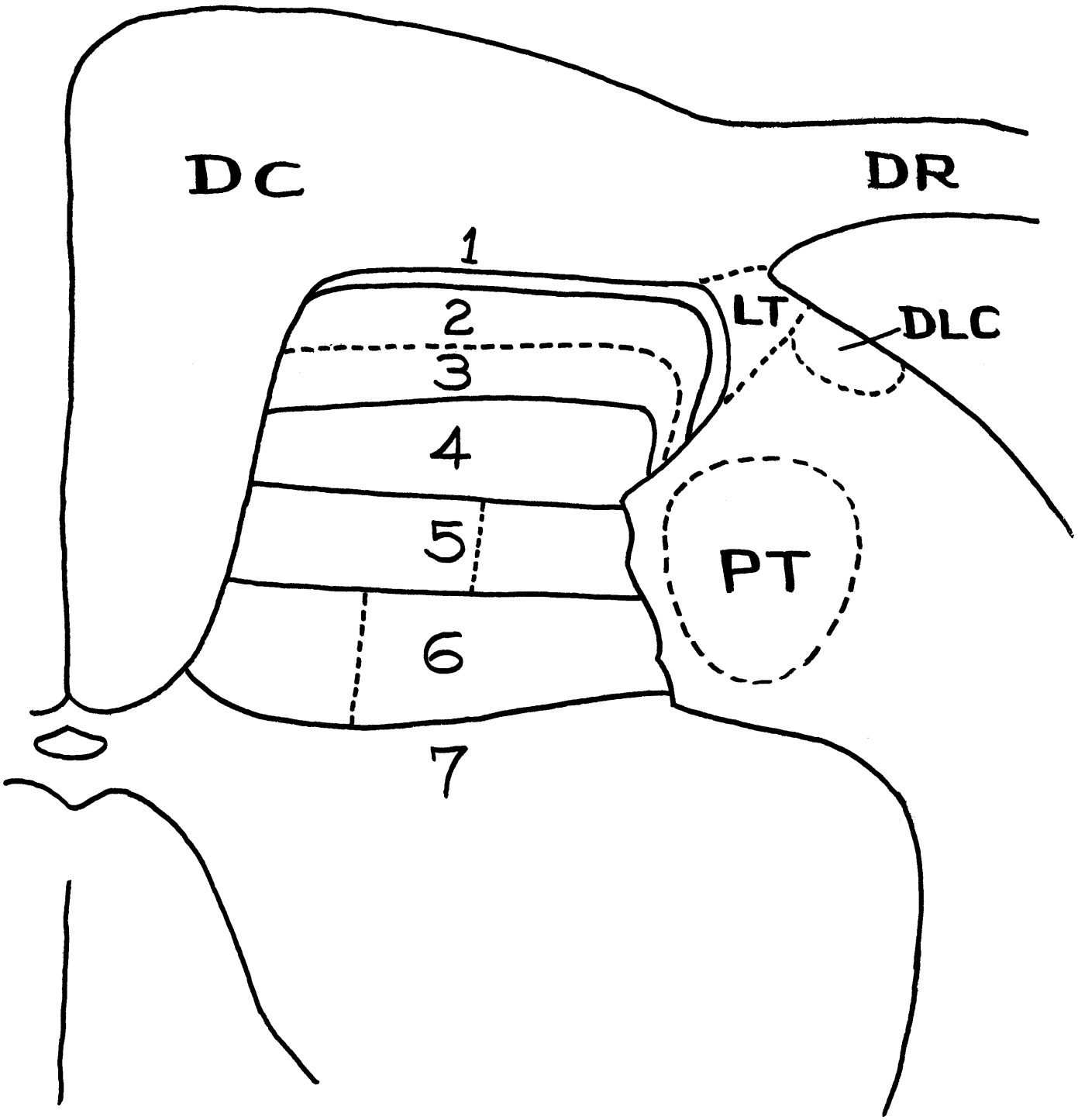


Figure 1. Rexed's laminar organization of the dorsal horn. Typical cross section of cat's spinal cord at the 7th lumbar level. Numbers label laminae distinguished by Rexed on the basis of cytoarchitectonic criteria. Abbreviations label fiber tracts: DC: dorsal columns, DR: dorsal root, LT: Lissauer's tract, DLC: dorsolateral column, PT: pyramidal tract.

layers 2 and 3: degeneration after dorsal root section was found only in layer 3 (but cf. Szentagothai, 1964b).

Using combined Golgi and degeneration techniques, Szentagothai (1964b) concluded that the axons of the layer 2 and 3 cells eventually terminate in other parts of this region; they may travel within these laminae, or via Lissauer's tract or via the lateral fasciculus proprius or may cross to the contralateral layers 2 and 3 via the posterior commissures. Thus, this region forms a self-contained system, with no outputs to other regions.

Lamina 4 contains a larger variety of cells, ranging in size from 8 x 11 μ to 35 x 45 μ, with the majority about 11 x 16 μ. The smaller cells tend to be rounded, with more cytoplasm than the layer 3 cells and little Nissl substance; medium cells are often triangular and contain more cytoplasm and Nissl substance, while the largest tend to be star shaped and contain considerable Nissl substance, sometimes in coarse granules. The dendrites of these cells arborize in layers 2 and 3, radiating perpendicular to the layer surface. Szentagothai (1964b) followed the axons of layer 4 and 5 cells in Golgi sections; these axons "with few exceptions" were found to enter the lateral fasciculus of the same side; none projected to the contralateral ventral white commissure, as previously supposed.

Lamina 5 cells are also highly variable in form and size, with the majority in the range 10-13 μ x 15-20 μ and extremes similar to layer 4. This lamina can be divided into two zones:

the lateral third of the layer is characterized by a reticulated appearance due to numerous longitudinally running fibers and by relatively more large cells containing much Nissl substance; the medial two-thirds contains more of the smaller cells, with less Nissl substance. In horizontal sections the bodies of the layer 5 cells tend to be oriented in a mediolateral direction (Wall, 1966). The dendrites of these cells have been found projecting as far dorsal as layers 2 and 3; their axons in part enter the lateral funiculus of the same side.

Lamina 6 is found in its typical form only in the cervical and lumbar enlargements; its cells range from 8 x 8 μ to 30 x 35 μ, with the majority in the range 10-12 μ x 12-15 μ. It can also be divided into a medial and lateral zone: the medial third contains more tightly packed and smaller cells, tending to have elongated shapes; the lateral zone has a looser arrangement of comparatively larger cells, tending to be triangular or star-shaped. The axons of some layer 6 cells cross in the ventral white commissures.

The two inputs to the dorsal horn via the dorsal root and the pyramidal tract have been studied histologically by degeneration techniques. Many collaterals of dorsal root fibers have been found to terminate in laminae 1 - 4 (Szentagothai, 1964a,b, Sprague and Ha, 1964). Of these the larger dorsal root fibers enter the dorsal columns in a medial direction, then curve ventrally around and through the medial portions of layers 1-3, traverse the lateral

part of layer 4 in a mediolateral direction and curve dorsally again to end in bushy, radially oriented arborizations in layers 2 and 3. The majority of fibers from a given dorsal root terminate in the same segment, although some have been found several segments above and below the root. The smaller fibers, in contradistinction to the large, send collaterals to the medial half of Lissauer's tract, where they ascend and descend up to three segments. From Lissauer's tract branches enter layer 1, forming a tangential plexus and synapsing with layer 1 cells; according to Szentagothai (1964b) most of these fine fibers turn ventrally to terminate in radial columns in layers 2 and 3.

The large afferent fibers also establish axo-somatic synapses with layer 4 cells. Sprague and Ha (1964) describe many larger fibers passing through lamina 4, some terminating in the medial regions of lamina 5 and others ending in the central parts of layer 6, the latter exhibiting dense degeneration.

Nyberg-Hansen and Brodal (1963) have recently re-examined the sites of termination of the PT fibers in the cat with reference to Rexed's laminae. After ablating portions of the pre- and postcruciate cortex, they examined various sections of the cord treated with silver impregnation methods of Nauta and Glees. After unilateral ablation of both pre- and postcruciate cortex most of the degenerating corticospinal fibers were found in the contralateral lateral funiculus (Fig. 1). These fibers entered the dorsal horn

through the lateral borders of laminae 5 and 6, then spread out in a fan-like fashion to laminae 4 - 7, where they "terminated" on the somata and dendrites of large and small cells. With lesions restricted to the precruciate "motor" cortex, degenerating fibers were mainly found in the lateral parts of layers 5 and 6, and the dorsal part of layer 7; a very few could be seen in the ventral region of layer 4. With lesions of the postcruciate "sensory" cortex, however, the degenerating fibers appeared throughout layer 5 and the medial area of layer 4, with a few appearing in the ventral part of layer 3 and the dorsal part of layer 6. Thus, a general segregation of PT fibers seems evident, those from the sensory cortex ending in the mediodorsal region of the horn, and those from the motor cortex in the ventrolateral region.

III. Physiology of dorsal horn interneurons.

Although many investigators have recorded from dorsal horn cells the first systematic characterization of the cells in various layers according to their responses to natural stimulation was attempted by Wall (1966). Particular recording and stimulating techniques were best adapted to establishing these characteristics, and the use of other techniques by earlier investigators probably explains their failure to obtain similar results. These techniques, used also in this study, include:

(a) extracellular recording with low impedance electrodes, which record fewer axons than fine-tipped electrodes; they also record more cells per track, making comparison of successive cells easier.

(b) checking each track histologically rather than relying on depth from surface or "standard outlines" of the cord.

(c) use of natural stimulation to characterize cells, rather than electrical stimulation of peripheral nerves, which drastically alters the response patterns of cells.

(d) use of decerebrate and spinal preparations rather using anesthesia, which suppresses the responses of many cells.

One disadvantage of these techniques is that less information is obtained concerning synaptic effects on cells from various sources than can be obtained by intracellular recording. It should also be pointed out that these methods preferentially select the largest cells for observation.

Using these techniques, Wall (1966) characterized the responses of cells in various regions of the dorsal horn. The few layer 1 cells observed seemed to respond to stimulation of large regions of the skin and sometimes muscle and joint movement, but were not extensively examined. The cells of layers 2 and 3 could only be characterized as too small to record from, although activity presumed to originate from layer 4 cell dendrites has been observed in this

region (Wall, 1965).

Layer 4 was the first region from which clear single cell responses could be repeatedly recorded; these cells typically responded to stimulation over a small area of the skin. Their receptive fields were larger than those of the afferent cutaneous fibers, indicating a convergence of input. Layer 4 cells at the rostral L7 level had receptive fields covering a fraction of a toe or a small area on the side of the foot. Receptive field in the proximal regions of the leg were larger than those found more distally. The layer 4 cells have been found to respond to bending of hairs, pressure, cooling, and chemical stimuli.

In lower regions of the dorsal horn, cells with much wider receptive fields were observed. The depth at which there was a transition from cells with small to cells with large receptive fields generally coincided with dorsal border of Rexed's lamina 5. Cells of this layer responded to skin stimulation over several toes or extensive regions of the foot and leg; these regions usually included the receptive fields of the immediately dorsal layer 4 cells. The threshold to natural and electrical stimulation of the skin often increased toward the periphery of the receptive field.

Cells in Rexed's lamina 6 were found to respond to movement of joints and pressure on muscles, as well as stimulation of the skin. In decerebrate cats almost all layer 6 cells had mixed cutaneous and proprioceptive fields; none responded to movements of joints other than those of the

ipsilateral leg. When the descending tracts from the brain stem were blocked by ice (i.e., the cat effectively rendered spinal) the cutaneous responses of almost all layer 6 cells increased, while the proprioceptive response of 2/3 decreased.

Wall also examined the effects of the PT on these cells by stimulating the medullary pyramids with continuous trains of .1 ms. pulses at 100/sec. He observed no PT effects on layer 4 cells, using intensities up to five times those required to evoke flexion of the hind limb (Wall, personal communication). Of 19 layer 5 cells examined, 6 were excited, 12 inhibited and 1 unaffected. Excitation was observed as an increase of firing during PT stimulation, a decrease of threshold of peripheral stimulation, and an increase of receptive field size; inhibition produced the converse effects. Of 19 layer 6 cells examined, 11 were excited, 7 inhibited and 1 unaffected. Responses to cutaneous and proprioceptive stimuli were affected the same way. The inhibited layer 6 cells often responded to the termination of PT stimulation with a vigorous discharge.

IV. Goals of the present investigation.

The present investigation was undertaken to obtain further information concerning the PT effects on dorsal horn interneurons, as characterized by their anatomical location and responses to natural stimulation. Wall's finding that no layer 4 cells were affected by the PT conflicts with the

reports of Carpenter, et al (1963) and Andersen, et al (1964) that cortical stimulation evoked presynaptic inhibition; a re-examination of this issue was clearly of interest.

Specifically, the goals of the present investigation include:

(a) observing and correlating the three types of responses to PT stimulation: the behavioral flexion response, the slow potentials evoked in the spinal cord, and the responses of single dorsal horn interneurons,

(b) locating the population of cells activated by the PT by a source density analysis of the PT-evoked field potentials,

(c) investigating the PT effects on afferent fibers by examining the PT-evoked dorsal root potentials, their interaction with peripherally evoked DRP's, and their possible correlation with decreased postsynaptic effectiveness of afferent volleys,

(d) characterizing the dorsal horn interneurons by layers and observing the PT effects on their spontaneous activity and their responses to peripheral stimulation, and

(e) investigating cells with axons ascending in the dorsolateral column, with respect to PT effects and anatomical location of their cell bodies.

METHODS

A.) Surgical Procedures.

Experiments were performed on healthy cats weighing over 2 kg. Under ether anesthesia the carotid arteries were ligated and the trachea cannulated. The head was clamped in a Horsley-Clarke stereotaxic apparatus in a flexed position, such that the line running from the tip of the nose to the tip of the jaw was 45° from the vertical.

The surgical preparation found most useful for the majority of experiments was similar to Lloyd's guillotine technique (Lloyd, 1941) and Preston's pyramidal preparation (Agnew, Preston and Whitlock, 1963). The cat was "spinalized" at the obex with a special transection leaving only the PT intact. This was accomplished with a blade shaped to sever all fiber tracts except the ventrally lying PT. After being "guillotined" with this blade the cat was artificially respirated. Nervous centers rostral to the section were destroyed anemically by occlusion of the vertebral arteries for ten minutes after guillotining, in addition to the previous ligation of the carotid arteries. Successful destruction of higher centers was gauged by loss of eye movements and blink reflex.

The PT stimulating electrode was then placed 6 - 10 mm. rostral to the obex section via a dorsal approach. The

electrode tip was considered to be in the right half of the PT when stimulation (0.4 ms. square pulses, 500/sec., continuous or 50 ms. trains) evoked flexion of the contralateral hind limb with intensities less than 1.5 volts (0.15 ma.), and when no responses of the ipsilateral hind limb were evoked by intensities up to 2.5 times this threshold. After optimum placement of the PT electrode, the exposed medulla and cerebellum were covered with cotton soaked in mineral oil (Nujol) and the skin reclosed with wound clips. This procedure seemed to retard edema of the exposed tissue and the consequent deterioration of PT responsiveness. After the experiment the guillotine section and PT electrode track were marked with india ink. The medulla was then fixed in formalin overnight and free-hand sections made to verify electrode position and extent of guillotine section. With the above placement procedure the electrode track was invariably found to terminate in the right half of the PT.

The preceding preparation had several advantages over alternative methods: (a) all non-PT descending pathways were destroyed by the section, (b) anemic decerebration caused less trauma than surgical decebration, and (c) nervous activity was not affected by anesthetia.

Several experiments were also performed with decerebrate preparations. The procedure described by Wall (1966) proved more satisfactory than the classical one. Our procedure differed only in substituting permanent ligation of

the carotid arteries for Wall's temporary occlusion. The PT electrodes were again placed as described above.

After recording thresholds for flexion responses to PT stimulation we paralyzed the animal with 1 cc. gallamine triethiodide (Flaxedil) i.v. Laminectomy from S1 to L4 was performed and the exposed spinal cord covered with warm mineral oil. Under magnified vision the dura was reflected from the midline and pinned to the surrounding muscle to form a "hammock" lifting the cord above the pulsating venous sinuses. Alternatively, the cord could be stabilized with several fine stainless steel rods placed crosswise under the dura and supported in grooves cut bilaterally in the vertebral pedicles.

A thermometer in the mineral oil bath monitored the cord temperature, which was maintained between 35° and 38°C. by means of an infra-red lamp applied intermittently. Additional warmth was continuously provided by a two-foot Chromalox radiation heater placed along the length of the animal. Flaxedil was administered in 0.5 - 1 cc. doses throughout the experiment whenever muscle twitching reappeared.

Most microelectrode recording was done at the rostral L7 level. The cord surface was usually prepared for microelectrode penetration as follows: a hot nichrome wire was held over the pia to cauterize dorsal blood vessels in the desired region, usually chosen to interfere with the minimum number of vessels. The hardened pia could then be slit

with a small wedge of razor blade.

B.) Stimulation Procedures.

The Tektronix 160 series waveform and pulse generators were used to produce pulse trains for PT stimulation and pulses at variable latencies for stimulation of skin, peripheral nerves, dorsal roots, or the dorsolateral column. Square pulses from the 161 generators were led to stimulus boxes designed by Dr. Karl Kornacker to deliver positive and negative pulses whose shapes could be independently controlled, to minimize recorded stimulus artifacts. These features were crucial when single cell responses had to be monitored during simultaneous stimulation of the PT.

The PT was stimulated with concentric bipolar electrodes; the inner electrode was a .01" insulated copper wire, surrounded by a 22 g. stainless steel cylinder (o.d. .031") insulated with Insulex. Both electrodes were exposed only at the tapered tip for a length of .3-.5 mm. and were separated by approximately .3 mm. Stimulation of peripheral nerves, dorsal roots or dorsolateral columns was done through well-chlorided Ag-AgCl hooks. Electrical stimulation of the skin was accomplished with two short 30 g. hypodermic needles placed into the superficial layers of the skin.

When natural stimulation was used to characterize dorsal horn neurons, no peripheral nerves or dorsal roots

were dissected (except for a few experiments in which the most caudal L6 rootlet was dissected for recording DRP's). The receptive field was found for each of three categories of skin stimulation: (a) brushing -- gently moving hairs with a #3 camel's hair brush, (b) touch -- resting a finger on the skin, and (c) pressure -- pinching a fold of skin with flat blunt forceps. Care was always taken to avoid moving the limb or pressing the muscles in producing cutaneous stimulation. A cell which increased its response to more intense skin stimulation and maintained a high rate of firing to continued pressure was characterized as having a "wide dynamic range". Dorsal horn cells were considered to have proprioceptive input from muscles and joints when they responded to gentle movement of the joints over small angles. Such movement invariably also stimulated some portion of the skin, but proprioceptive responses could usually be distinguished from cutaneous by being more regular and sustained for maintained joint angles, and by adding to any responses to deliberate cutaneous stimulation.

C.) Recording.

Dorsal horn field potentials and extracellular single neuron responses were recorded with glass micropipettes filled with 3M KCl and having a tip impedance of about 1 Megohm. The tips of such electrodes could be left near single cells for hours without altering their responses,

indicating negligible leakage of KCl into the cord. A micrometer advanced the electrode into the cord in a vertical direction. To record from axons in the dorsolateral column unbroken glass micropipettes with impedances of 10 - 30 Megohms were used.

Dorsal root potentials (DRP's) were recorded from the smallest convenient caudal rootlet of the L6 dorsal root. A pair of Ag-AgCl hooks was used, one placed under the rootlet as close as possible to the cord without touching it, the other under the distal, crushed end of the rootlet.

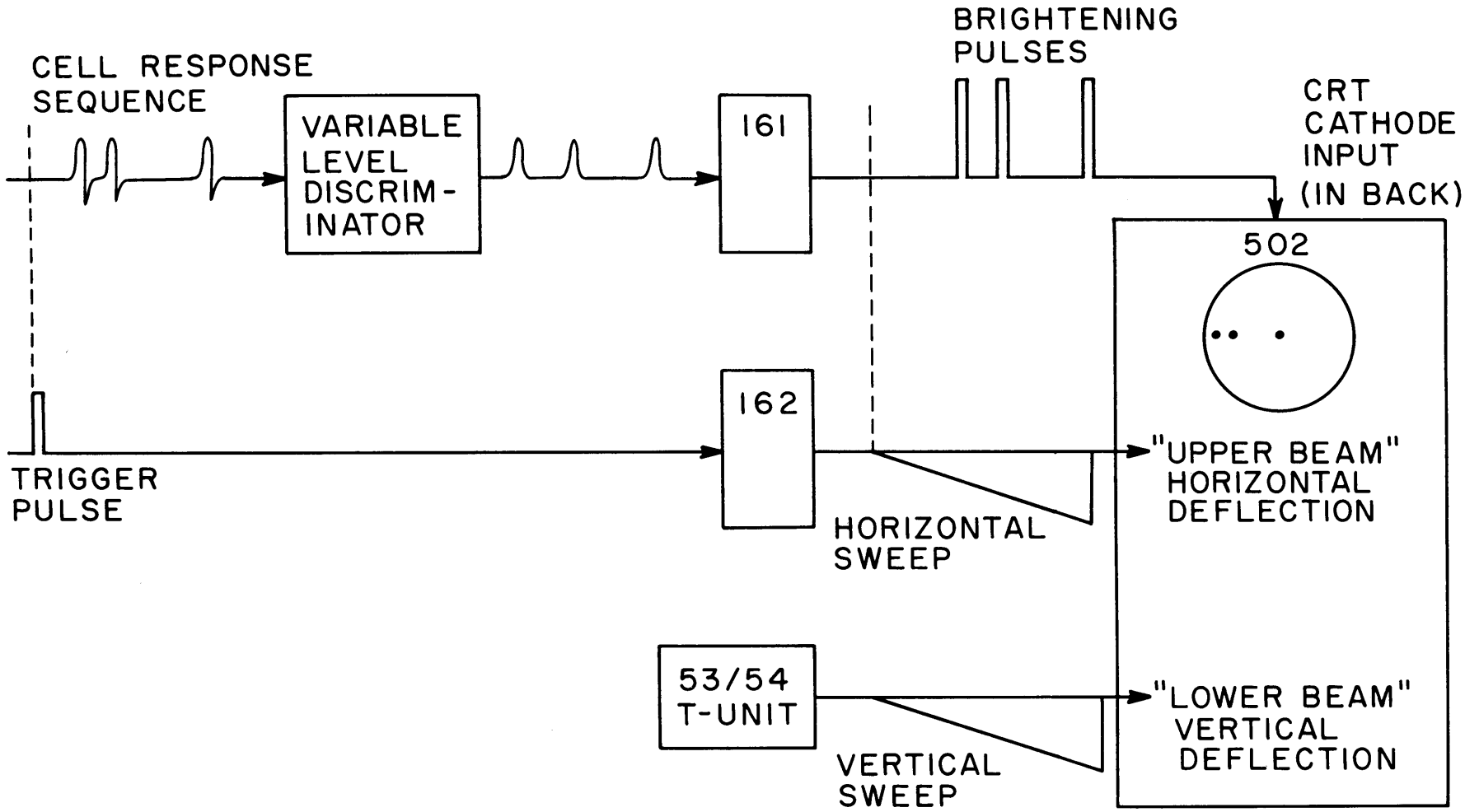
Signals from the microelectrode and an indifferent lead on the animal, or from the two Ag-AgCl hooks were fed into a double cathode follower headstage, then to a differential amplifier (Tektronix E-unit) whose output was displayed on a Tektronix 502 oscilloscope. For DC recording of maintained slow potentials the cathode follower output could be led directly to the 502, operated in DC mode. In most situations slow potentials could be recorded without distortion with the E-unit bandwidth set at 0.2 - 10^3 c.p.s. Single cell responses were recorded at 80 - 10^4 c.p.s., with a .05 uf capacitor placed between the electrode and cathode follower input to eliminate any DC grid current. All varieties of responses could be recorded on a 4-channel Ampex SP-300 tape recorder.

A convenient method of displaying single unit responses called the "raster" display was used to exhibit in compact form the responses of a cell to repeated stimuli. Instead

of displaying the sequence of action potentials of the cell, we converted these to a row of dots, preserving only the time sequence of firings in the spacing of the dots. Responses to successive stimuli are displayed in adjacent rows; thus any variability in the response becomes apparent. This method also clearly demonstrates the interaction of two different stimuli -- peripheral and PT -- by displaying the response to each given separately and both given simultaneously.

The procedure for producing a raster display is diagrammed in Fig. 2. As shown, the display is produced on the face of a Tektronix 502 oscilloscope, whose operation is altered in two ways: (a) the CRT cathode input is set to receive voltage pulses, which produce bright dots on the screen, and (b) the upper beam input is connected to the horizontal deflection plates so that horizontal sweeps can be generated by a sawteeth from a 162 waveform generator. This 162 is triggered by a timing pulse preceding each stimulus by a fixed interval. The sequence of cell action potentials is converted to a sequence of brightening pulses as shown. A Tektronix 53/54 T-unit generates a slow sawtooth of up to 10 seconds duration which controls the vertical deflection plates, so that successive horizontal sweeps are displayed beneath each other on the screen. PT stimulus trains and calibration pulses are converted to dots in a similar way. The whole display can be photo-

Figure 2. Circuit for producing raster display. All numbers refer to Tektronix equipment. As shown, a sequence of cell responses was converted to square pulses which brightened the beam of the 502 oscilloscope. The horizontal deflection of the beam was generated by a 162 waveform generator triggered by a pulse preceding each stimulus - response sequence. This produced a horizontal row of dots for each response sequence; succeeding sequences were displayed in vertically displaced rows. The separation of adjacent rows was controlled by a slow sawtooth from a 53/54 T-unit (a plug-in time-base generator from a 536 oscilloscope). Further discussion in text and Fig. 13.


CELL RESPONSE SEQUENCE
VARIABLE LEVEL DISCRIM-INATOR
161
BRIGHTENING PULSES
CRT CATHODE INPUT (IN BACK)
502
162
TRIGGER PULSE
"UPPER BEAM" HORIZONTAL DEFLECTION
HORIZONTAL SWEEP
53/54 T-UNIT
"LOWER BEAM" VERTICAL DEFLECTION
VERTICAL SWEEP

graphed with a Polaroid camera.

D.) Histological verification of electrode position.

Since it was important to determine the location in the dorsal horn of cells whose responses had been recorded, and since the shapes of spinal cords vary significantly, the positions of electrode tracks had to be verified in each experiment. This was done by cutting off the tips of the electrodes in the spinal cord and preparing a section containing the electrode for direct observation. After advancing the electrode to a known depth (usually 3 mm.) the tip was cut under magnified vision with a special pair of fine dissection scissors, having a notch in one blade. With the electrode stem resting in the notch just enough pressure could be exerted to crush the glass; this usually left the severed tip and the stump of the electrode visibly aligned. At the end of the experiment the cord was fixed *in situ* with 10% formalin for at least 30 minutes; then a large section of cord containing the microelectrode was removed and further fixed in 10% formalin for at least 24 hours. A 1 mm. thick slab of cord containing the electrode was then cut freehand and observed under the microscope. In most cases the depth of the electrode in the section agreed with the micrometer depth to within 5%.

RESULTS

I. Behavioral Response.

A. Parametric variations.

Stimulating the medullary PT of a guillotined or decerebrate cat evoked flexion of the contralateral hind limb; often the contralateral forelimb responded as well. Threshold stimulus intensities were taken to be the smallest intensities which repeatedly evoked a visible twitch of the muscles. When the PT electrode was properly placed threshold intensities were usually found to be between 0.5 and 1.5 volts (.03 - .15 ma.), for long trains of square pulses of 0.4 ms. width and 500/sec. frequency. Thresholds for 50 ms. trains and continuous stimulation were both taken regularly; at 500/sec. these were often the same, and never differed by more than 20% (Fig. 3). The stimulus intensities at which muscle movement could just be discerned served as a useful level against which to compare intensities for evoking other responses, and will be subsequently referred to as "flexion threshold". Higher stimulus intensities evoked correspondingly more vigorous flexion, until at about three times threshold the animal responded with an over-all massive jerk of the body. With proper electrode placement ipsilateral limbs showed no response for intensities up to 2.5 times threshold.

A minimum of 3-4 PT shocks was generally necessary to evoke any movement; the vigor of response increased with train length up to about 50 ms. Continuous stimulation usually evoked alternate flexion and relaxation, although tonic flexion was also observed. As would be expected hyperbolic strength-duration curves could be obtained; Fig. 3 shows an example from a decerebrate preparation.

The frequency dependence of the flexion response was remarkably regular from one cat to the next. Fig. 4a shows a typical variation of flexion threshold with continuous stimulation and .4 ms. pulse width. Since the most effective frequency for all types of responses was invariably in the range 400-600/sec., a standard frequency of 500/sec. was chosen for later experiments and will be implied whenever frequency is not explicitly given.

The pulse width used in most experiments was .4 ms. Responses generally decreased with smaller widths, but did not increase with wider pulses.

B. Interaction with peripheral stimulation.

When the skin of the hind leg was brushed or touched during PT stimulation the threshold for movement was considerably reduced. Therefore all measurements of flexion threshold were made after at least one minute's rest.

If 50 ms. PT trains of just suprathreshold intensity were repeated every 1 to 2 seconds, the muscle twitch fre-

Figure 3. Threshold for evoking flexion as a function of PT train length. These intensity - duration curves were obtained for two degrees of response: (a) threshold for evoking the slightest visible muscle twitch ("flexion threshold"), and (b) threshold for withdrawal of foot by one inch. Stimulus frequency was 500/sec.; pulse width 0.4 ms. Intensities were measured in terms of current; the voltage scale was determined later. Above 0.2 ma. = 1.75 volts stimulus current and voltage were linearly related.

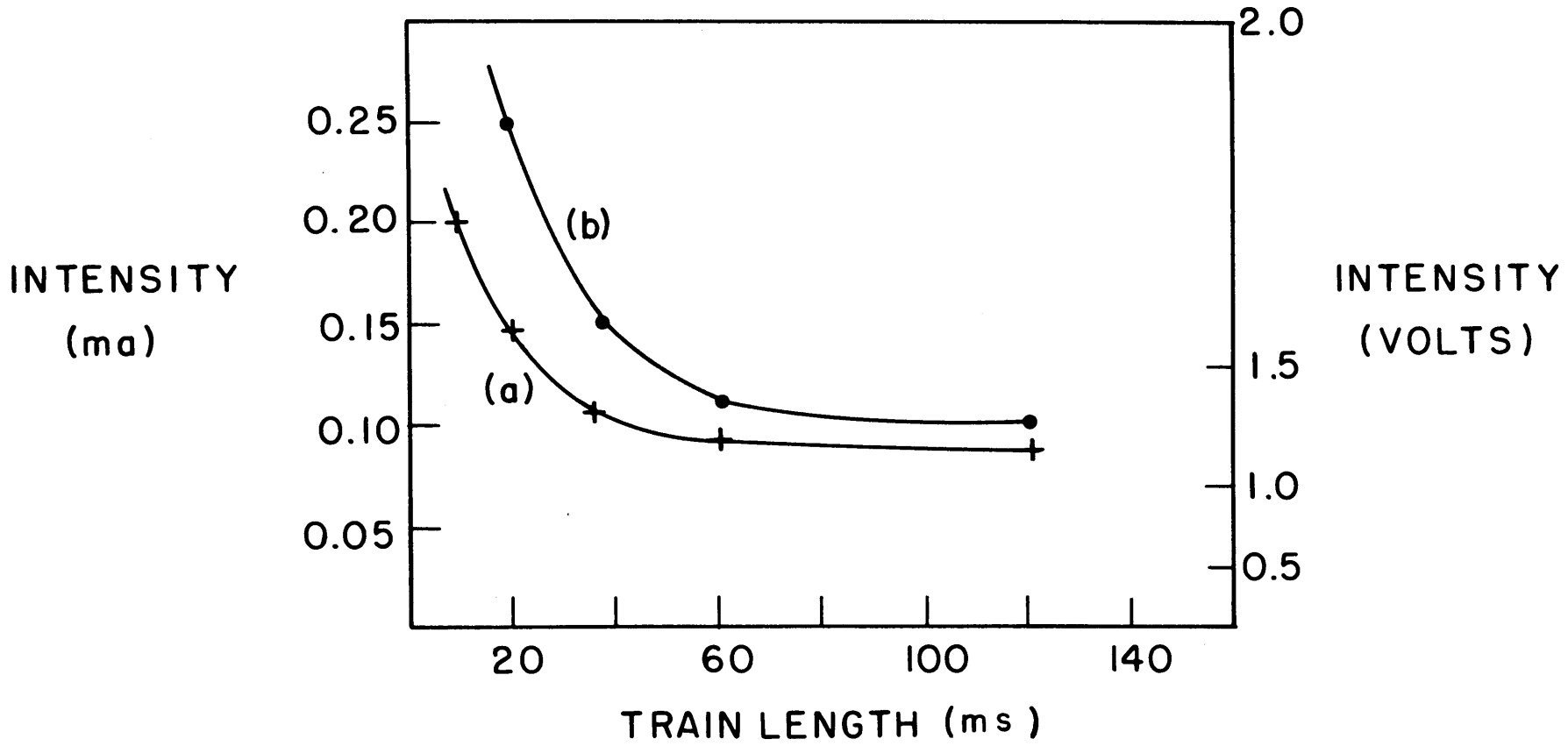
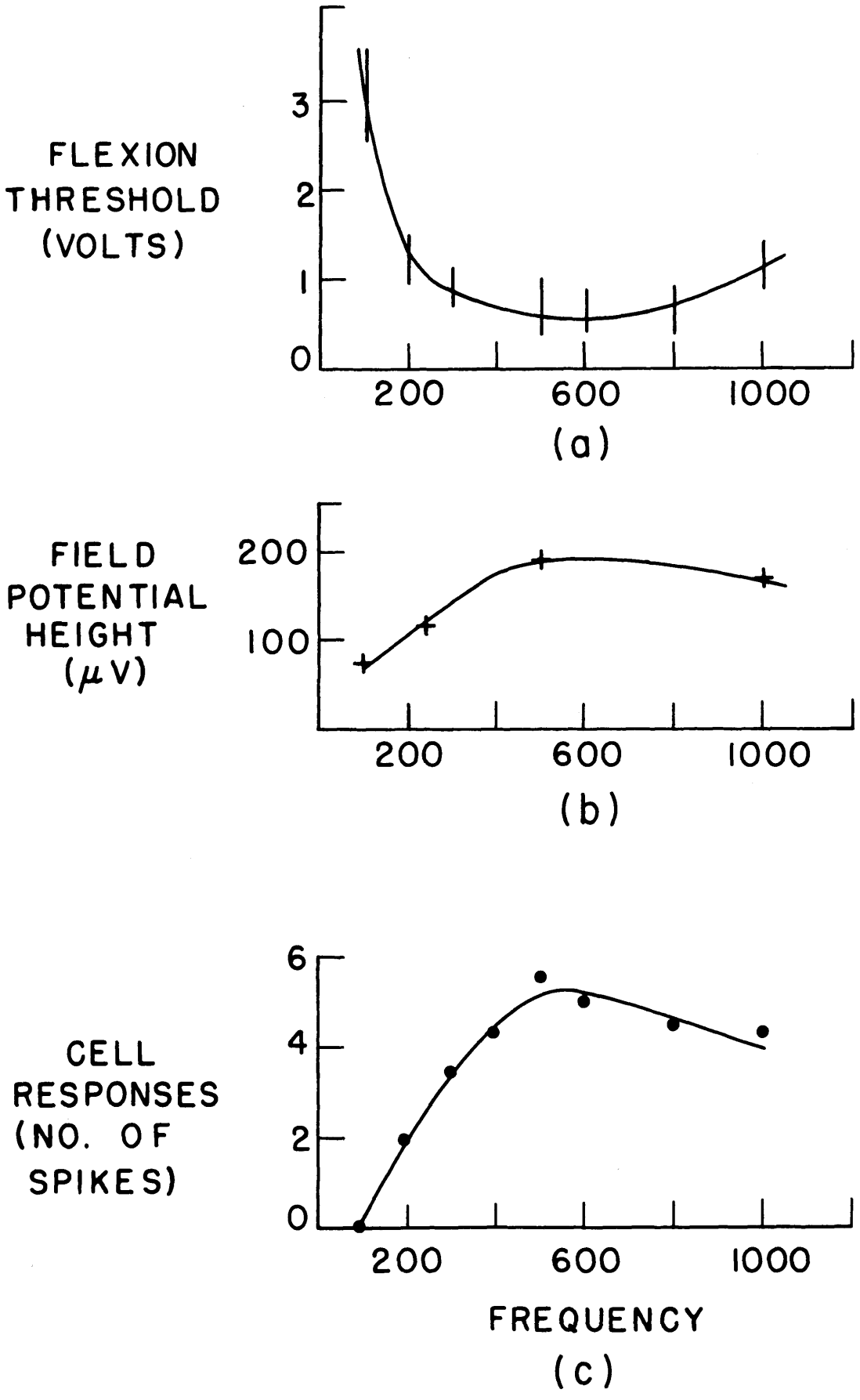

INTENSITY
(ma)
0.25
0.20
0.15
0.10
0.05
(b)
(a)
2.0
1.5
1.0
0.5
INTENSITY
(VOLTS)
20
60
100
140
TRAIN LENGTH (ms)

Figure 4. Variation of responses with PT frequency. Pulse width was 0.4 ms.

(a) threshold for visible muscle twitch in contralateral hindlimb with continuous stimulation ("flexion threshold").

(b) height of field potentials evoked by 10 PT shocks, at depth of 2 ms. in lumbar spinal cord.

(c) average number of responses of single cell to 100 ms. PT train. Recorded in the dorsolateral column.


FLEXION THRESHOLD (VOLTS)
3
2
1
0
200
600
1000
(a)
FIELD POTENTIAL HEIGHT (μV)
200
100
200
600
1000
(b)
CELL RESPONSES (NO. OF SPIKES)
6
4
2
0
200
600
1000
FREQUENCY
(c)

quently disappeared after the third or fourth repetition. Touching or moving the leg would bring the responses back; when peripheral stimulation ceased, the response again disappeared within several repetitions. As seen below, this "habituation" of responses to PT stimulation was relatively uncommon in the responses of dorsal horn neurons and would probably arise from effects in the ventral horn.

II. Slow Potentials

By slow potentials we mean extracellular potential changes in the frequency range 1-10^3 c.p.s., generated by a population of neurons. We were interested in two types: the field potentials recorded in the dorsal horn and produced by the activity of spinal cord cells, and the dorsal root potentials recorded from a dorsal rootlet and produced by the depolarization of the afferent fibers. The field potentials were of primary interest in locating the groups of cells activated by PT stimulation; toward this end a method of analyzing these potentials to derive their sources was employed. The dorsal root potentials were useful indicators of the state of depolarization of afferent fibers.

A. Field Potentials

1. Relation of field potentials and their generators.

Stimulating the PT or skin electrically activates a

population of neurons in the spinal cord. This activity generates extracellular potentials by (a) producing ionic current flows in a resistive medium, (b) causing local changes in conductivity due to alterations in membrane resistances, (c) producing local dipole fields due to depolarization of active neurons, and possibly (d) generating local electromagnetic fields due to rapid current changes. The net result of such events in a population of simultaneously active neurons is the field potential recorded in the extracellular medium. To relate the shape of this potential to the spatial configuration and temporal firing pattern of the neurons is hopeless without invoking a substantial set of simplifying assumptions. The most one could hope to learn from the field potentials is the location of the generating cells.

By systematically recording the slow potentials over a region of the nervous system, people have attempted to locate the regions of activated cells by seeing where the potential is largest (Coombs, Curtis and Landgren, 1956). Unfortunately the size of the potential at any point is not an accurate index of the local density of field generators because this potential includes the effects of distant activity. The distance over which potentials drop off in the passive medium is usually large compared to the dimensions of active regions; thus, the spatial resolution of this method is simply inadequate to resolve most cell popu-

lations. The problem becomes critical when two nearby but separate regions of activity are to be distinguished. Consider for example the situation if two nonadjacent layers of the spinal cord -- e.g., laminae 4 and 6 -- were to be simultaneously activated by PT stimulation. An electrode would record large potentials between these layers, where no cells were active.

To obtain a more useful index of the local density of field generators one would have to exclude the effects of distant activity. One such index subtracts from the potential at any point the average of the potentials at surrounding points. For example, if potentials $\Phi(\vec{r})$ are recorded at points regularly spaced in a three-dimensional Cartesian grid, then this index of the local density of field generators would be

$$D(\vec{r}) = \Phi(\vec{r}) - \frac{1}{6}\sum_{i=1}^{6} \Phi(\vec{r} + \vec{a}_i) \qquad \text{(II-1)}$$

where $\vec{a}_i$ represents a unit vector from the point $\vec{r}$ to one of its six nearest neighbors along the major axes. Any contribution to the potential arising from generators outside this region will be the same for the center point and for the average of the six surrounding points, and will therefore vanish from the total expression.

This formula is actually the first order approximation to the Laplacian of Φ , a common expression for the "source density" of any scalar field Φ . An alternative derivation

of the formula begins with this general expression for the source density of a field. Although the forms of such expressions are identical for a large variety of fields (Morse and Feshbach, 1953) probably the most common example is the relation between the charge density ρ and its electrostatic potential field φ :

$$\rho(\vec{r}) = -\nabla^2 \varphi(\vec{r}) \qquad \text{(II-2)}$$

By analogy, a similar expression can be assumed to give the local density of neuronal sources of the physiological "field potentials". Although the physiological situation involves time-varying fields, the analogy is justified if the potentials are all taken at the same latency after stimulation. The physiological situation also involves several types of neuronal events which contribute to the field potentials; however, for the purposes of locating the active neurons, all these generating mechanisms can be lumped together without explicitly calculating their individual contributions. (One model, proposed by Howland, et.al. (1955) and Kornacker, (1963) assumes the only contribution to the field potentials to be ionic currents in the extracellular medium and neglects resistance changes. Under these circumstances the local current density $\vec{J}$ is related to the electrical field vector $\vec{E}$ by the (constant) conductivity σ : $\vec{J}=\sigma\vec{E}$. $\vec{E}$ is assumed to be related to the field potentials ϕ by the electrostatic expression $\vec{E}=-\nabla\phi$. The combination of these ex-

pressions yields for the local density of current sources: $S \equiv \nabla \cdot J = -\sigma \nabla^2 \Phi$. This model, then, identifies the field generators with current sinks.)

Assuming the Laplacian expression to give the source density of the field potentials, we can calculate its first order approximation. The method is illustrated most simply for the one dimensional case:

$$\rho(x) \equiv -\nabla^2 \Phi(x) = -\frac{\partial^2}{\partial x^2} \Phi(x)$$

$$\approx -\frac{\Delta}{\Delta x}\left(\frac{\Delta \Phi}{\Delta x}\right)$$

$$= -\frac{\Delta}{\Delta x}\left[\frac{\Phi(x+a) - \Phi(x)}{a}\right]$$

$$= -\frac{1}{a}\left[\frac{\Phi(x+a) - \Phi(x)}{a} - \frac{\Phi(x) - \Phi(x-a)}{a}\right]$$

$$= \frac{2}{a^2}\left[\Phi(x) - \frac{1}{2}\sum_{i=1}^{2} \Phi(x + a_i)\right]$$

Where Δ denotes a small difference and $a \simeq \Delta x$ is a small distance and a_i is the one-dimensional correlate of the vector $\vec{a}_i$ defined for eq. II-1. For three dimensions this expression is easily generalized to

$$\rho(\vec{r}) = \frac{6}{a^2}\left[\Phi(\vec{r}) - \frac{1}{6}\sum_{i=1}^{6}\Phi(\vec{r}+\vec{a}_i)\right]$$

The term in brackets is clearly identical to the right-hand side of expression II-1. Thus, taking the second spatial derivative of the potential gives the physical quantity we are seeking -- the density of field generators -- and automatically increases the spatial resolution with which this quantity is determined.

The resolution is determined by the separation, a, of the recording points. This distance should be large compared to the size of individual neurons, but smaller than the dimensions of the neural population to be distinguished. In the spinal cord, where the laminae are about $\frac{1}{2}$ mm. thick, a useful grid size is 150 μ. This should resolve any sources to within 300 μ.

2. PT Field Potentials

Examples of field potentials evoked in the dorsal horn by PT stimulation are shown in Figs. 5 and 6. Above flexion threshold intensities, a 10 ms. train of PT shocks produced a negative potential reaching a peak about 22-25 ms. after the initial PT shock (Fig. 5a). This potential could be recorded throughout the dorsal horn and dorsal columns, but was largest in layers 5 and 6. It increased with stimulus intensities, saturating at about 4 times flexion threshold.

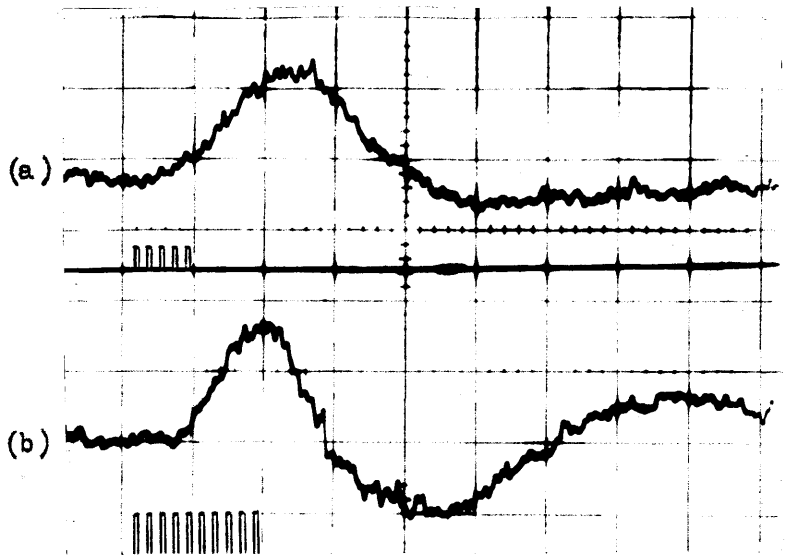


Figure 5. Field potentials evoked by PT stimulation. Recorded at depth of 1.5 mm. in the spinal cord illustrated in Fig. 6.

(a) negative wave evoked by low intensity PT stimulus: 5 shocks at 0.6 volts.

(b) negative and positive waves evoked by higher intensity PT stimulus: 10 shocks at 2 volts.

PT stimulus trains are shown below each response. The variation with depth of the negative wave was the same at both intensities and is further illustrated in Fig. 6.

Vertical calibration for field potentials: 100 μV./div.
Horizontal calibration: 10 ms./div.

In addition, more intense PT stimulation generally evoked a later positive wave, which was large in the dorsal columns and decreased with depth, reversing in the region of layer 6. (Figs. 5b,6). Long PT stimulus trains of 100 ms. or more often evoked this same positive wave after the initial negative phase, but sometimes prolonged the negative potential. Due to the variability of this later phase and the fact that it must represent more complex polysynaptic activity, it was not further interpreted. The early negative wave, however, was regularly observed and was taken to reflect initial responses to PT stimulation. The height of this negative wave varied with PT frequency in the same way as the flexion response (Fig. 4b).

The spatial variation of the PT field potentials with depth is shown in Fig. 6. Typically, the height of the negative wave was constant from the cord dorsum to around layer 4, then it increased to peak in layer 5 or 6, then rapidly decreased again. At a given depth its variation in the rostrocaudal direction was undiscernable over distances of 5 mm.; its variation in the mediolateral direction was also negligible (an increase of 10% /mm. in the lateral direction). By far the greatest spatial variation was in the dorsoventral direction (about 300%/mm. in active regions). This accorded with the laminar arrangement of cell groups found anatomically, and justified using a simpler one-dimensional first order approximation to calculate the source densities.

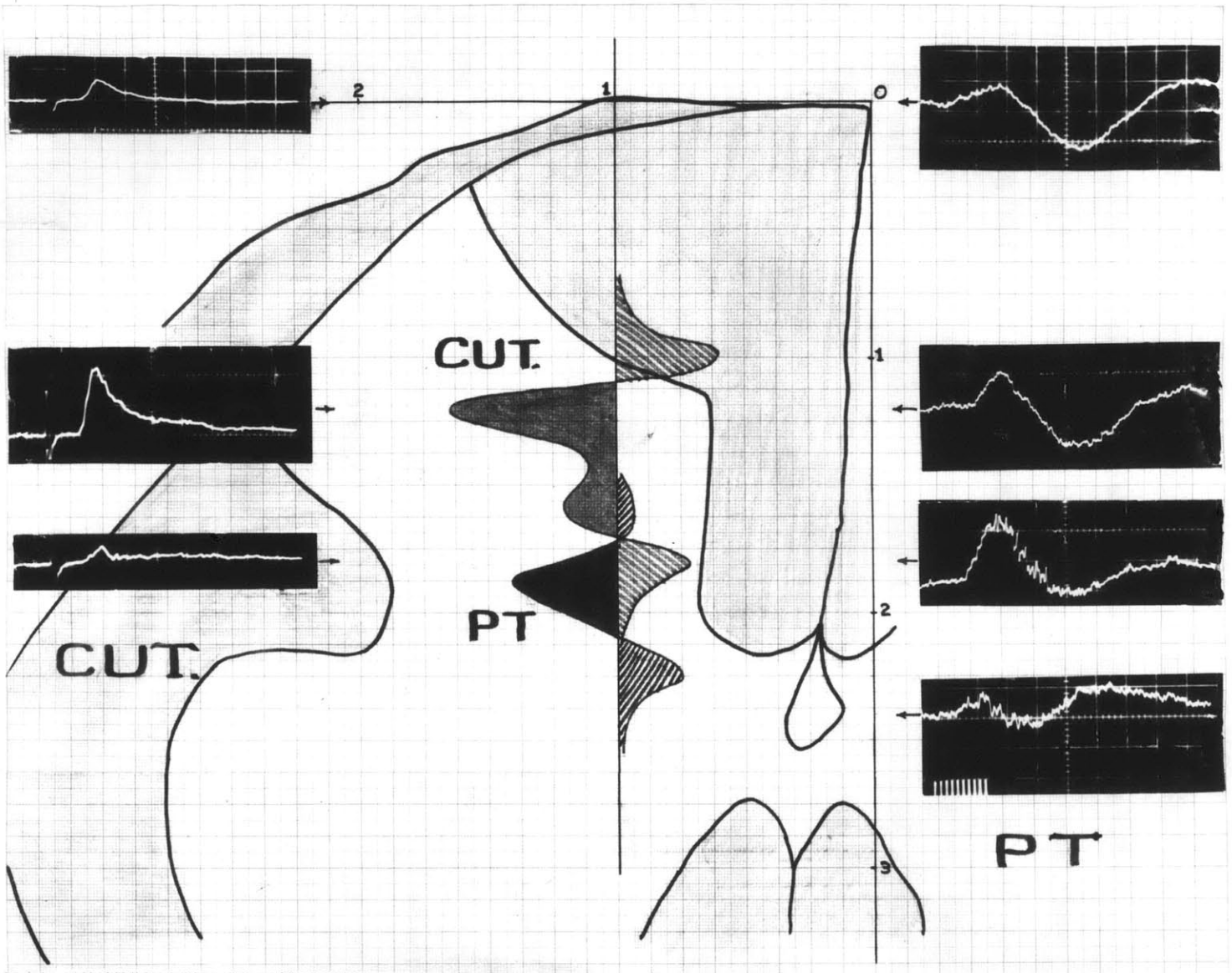
Figure 6. Source density of field potentials evoked by stimulation of PT and skin.

Cross-section of spinal cord at rostral L7 is shown with vertical electrode track 1 mm. lateral to midline. Along the track is plotted the source density distribution of the field potentials, as calculated from equation II-1.

On the left (labeled "cut.") are examples of the field potentials evoked by a 0.4 ms., 4 volt shock to the skin of one toe, and shown at the depth of recording. These potentials had the greatest source density in layers 2 - 5, as shown by the distribution labeled "cut."

On the right (labeled "PT") are examples of field potentials generated by PT stimulation (10 shocks, at 2 volts and 0.4 ms. pulse width and 500/sec. frequency); arrows indicate the depth of recording. The PT source density was greatest in layer 6 and the ventral part of layer 5, as shown by the lower distribution plotted along the electrode track.

The field potentials evoked by skin stimulation were about ten times larger than those evoked by the PT, so the scale of the "cutaneous" source density was reduced by ten with respect to that of the PT source density. On the field potential plots the horizontal scale is 10 ms./div.; the vertical scale is 1 mV./div. for "cut.", and 100 μV./div. for PT.


2
1
0
CUT.
1
CUT.
PT
2
PT
3

In the experiment illustrated in Fig. 6 a vertical track was made 1mm. lateral to the midline at rostral L7. At 150μ intervals recordings were made of field potentials evoked by two intensities of PT stimulation (as in Fig. 5), and by electrical stimulation of the skin. Five examples of each response were tape recorded, later displayed on an oscilloscope screen and visually averaged. From the plot of potentials as a function of depth, the source density was computed, using formula II-1. The source densities for skin and PT stimulation are shown in Fig. 6 plotted along the electrode track.

The initial negative wave evoked by the two intensities of PT stimulation had the same distribution; both showed strong sources in layer 6 and the ventral part of layer 5. In contrast, the skin stimulation evoked potentials with sources in layers 3 - 5. From one cat to the next the exact shapes and widths of these distributions varied somewhat. Their invariant feature was that the sources of the PT-evoked field potentials were in the ventral portion of the dorsal horn (layers 5 and 6), while skin stimulation produced the greatest source density in the dorsal half (layers 3 to 5).

B. Dorsal Root Potentials.

Dorsal root potentials (DRP's) are closely correlated with a depolarization of the afferent dorsal root fibers. This depolarization is thought to be generated near the

termination of these fibers and to spread electrotonically into the dorsal root, where it can be recorded as a potential difference between two points on the dorsal root. This depolarization has been shown to be correlated with a decrease of post-synaptic effectiveness of impulses arriving over the afferent fibers -- a phenomenon called "presynaptic inhibition".

Examples of DRP's evoked by PT stimulation are shown in Figs. 7-10. Several PT shocks were usually necessary to produce a visible DRP. The typical DRP evoked by a PT stimulus train of 50 ms. or less was a negative wave beginning at about 20-25 ms. after the initial shock, rising to a maximum around 50-60 ms. and decaying to zero at 100-125 ms. A smaller positive phase often appeared for 100 ms. after the negative. The DRP appeared at stimulus intensities below flexion threshold and increased with intensity (Fig. 7). PT trains of 100 ms. or longer produced the negative peak at 60 ms., and maintained the DRP at a plateau level for the remainder of the stimulus (Fig. 8); the height of this plateau varied between the height of the original peak and zero.

Peripheral stimulation also produced DRP's, which interacted with those evoked by the PT. Continuous brushing of the skin of the hind limb evoked a tonic DRP of 300-400 μV. for the duration of the stimulus. This tonic peripheral DRP very effectively occluded the negative PT-DRP. (Fig. 9).

Electrical stimulation of the skin evoked a sharply

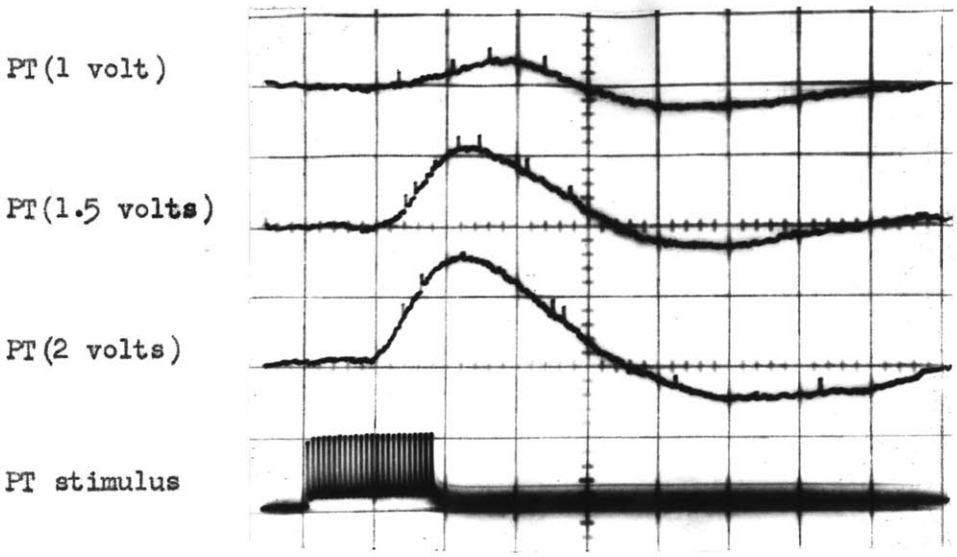


Figure 7. Dorsal root potentials evoked by a brief PT train.

The PT was stimulated for 45 ms. at the different intensities shown above. The DRP's exhibited negativeand positive phases for all intensities. Flexion threshold was 0.7 volts.

The DRP's shown here and in all subsequent figures were recorded from a caudal L6 dorsal rootlet. Vertical scale for DRP's: 200 μV./div.; horizontal scale: 25 ms./div.

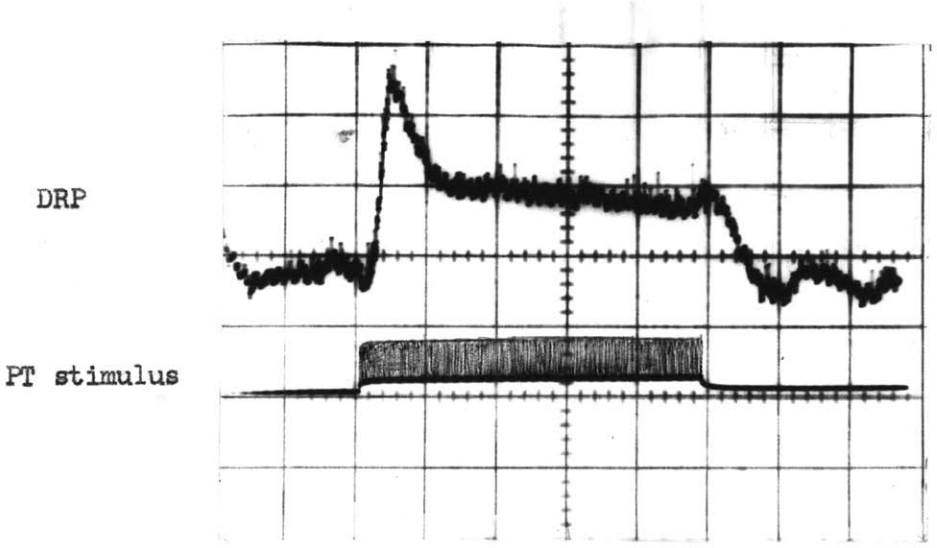


Figure 8. Dorsal root potential evoked by a long PT train.

After an initial peak the DRP remained at a plateau level for the remainder of the PT stimulus. The height of this plateau varied between animals, from the height of the initial peak to zero. However, even when the DRP returned to zero during long PT trains, there was a reduction in postsynaptic effectiveness of afferent volleys (cf. Figs. 11 and 14).

The 500 ms. PT train is shown below DRP. PT intensity was 1.6 volts (2 times flexion threshold).

Vertical scale for DRP: 100 μV./div.; horizontal scale: 100 ms./div.

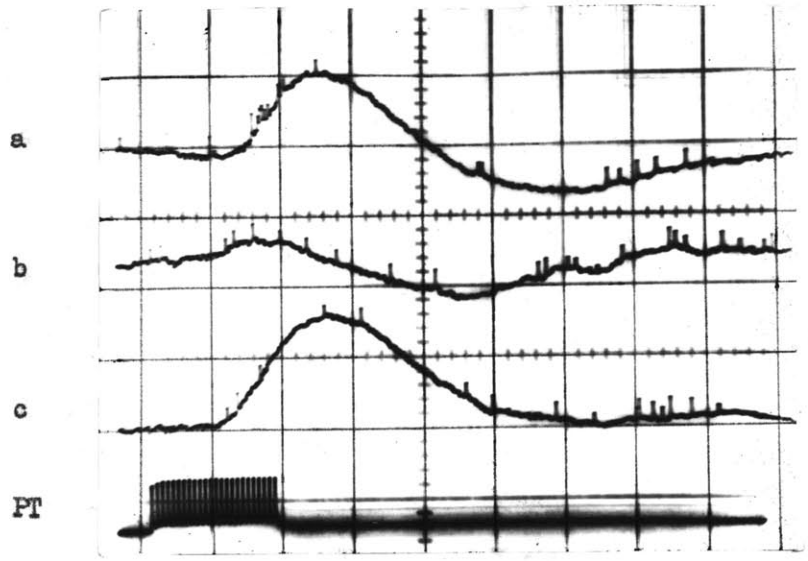


Figure 9. Occlusion of the PT-evoked DRP by a tonic peripherally evoked DRP.

A continuous touch of the leg evoked a tonic negative DRP of 300 μV. Here, this tonic DRP was superimposed on the biphasic PT-DRP to see whether the positive phase could be enhanced, as can happen with a biphasic peripherally evoked DRP (Mendell and Wall, 1964). Figure shows the PT-DRP (a) before, (b) during, and (c) after the tonic negative DRP evoked by touch. Although the negative phase of the PT-DRP was reduced during the touch, there was no enhancement of the positive phase, indicating that the latter probably does not represent hyperpolarization of the afferent fibers. Immediately after the touch was released (c), the negative phase was enhanced.

The PT stimulus was a 45 ms. train at 1.5 volts (2 times flexion threshold). Vertical scale: 200 μV./div.; horizontal scale: 25 ms./div.

rising DRP lasting 50-75 ms. in these unanesthetized preparations (Fig. 10a). A conditioning PT train reduced this DRP to an extent not explainable by occlusion alone (Fig. 10b). The reduction clearly involved an inhibitory process -- the most likely being presynaptic inhibition. If the PT depolarized the afferent fibers, any volleys coming into the cord over these fibers would be less effective in evoking postsynaptic activity, including that activity involved in producing a DRP. This is discussed further in a later section.

A measure of the reduction of the peripheral DRP by preceding PT stimulation is given by the expression:

$$R(\tau) = \frac{V(PT) + V(e.s.) - V(PT+e.s.)}{V(PT) + V(e.s.)} \times 100 \quad \text{(II-3)}$$

where V(PT) = height at latency τ of DRP evoked by PT

V(e.s.) = " " " " " " " " electrical stimulation of skin

V(PT+e.s.) = " " " " " " " " " + PT

In the experiment of Figs. 10 and 11 this reduction was determined at various latencies, where "latency" meant the time between the first PT shock and the peak of the negative wave of the peripherally evoked DRP. When the PT was concontinuously stimulated up to the peripheral DRP (Fig. 10), the reduction was evident long after the PT-DRP had returned to zero.

For comparison the series was repeated with a short PT train, as shown in Fig. 12. Here the peripheral DRP was

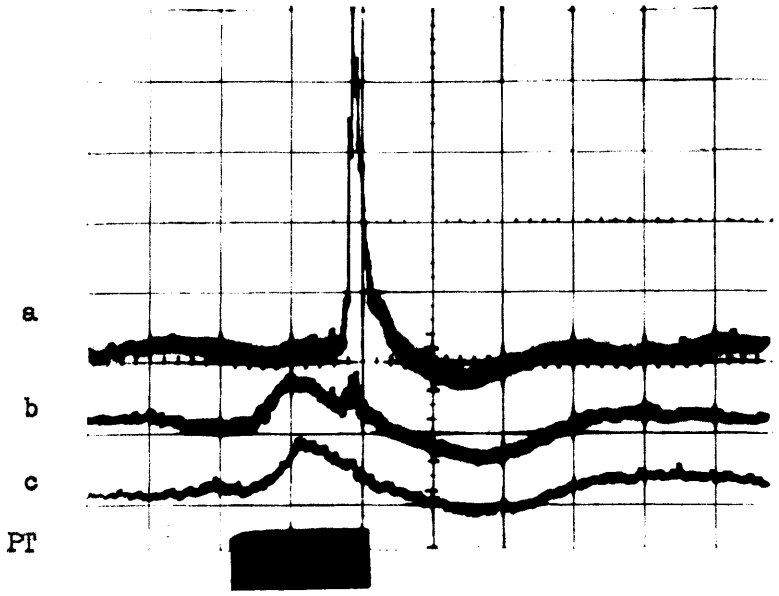


Figure 10. Reduction of a peripherally evoked DRP by a long conditioning PT stimulus.

(a) DRP evoked by single shock to skin of toe.

(b) DRP evoked by shock to toe, preceded by a 100 ms. PT conditioning train.

(c) DRP evoked by 100 ms. PT train alone.

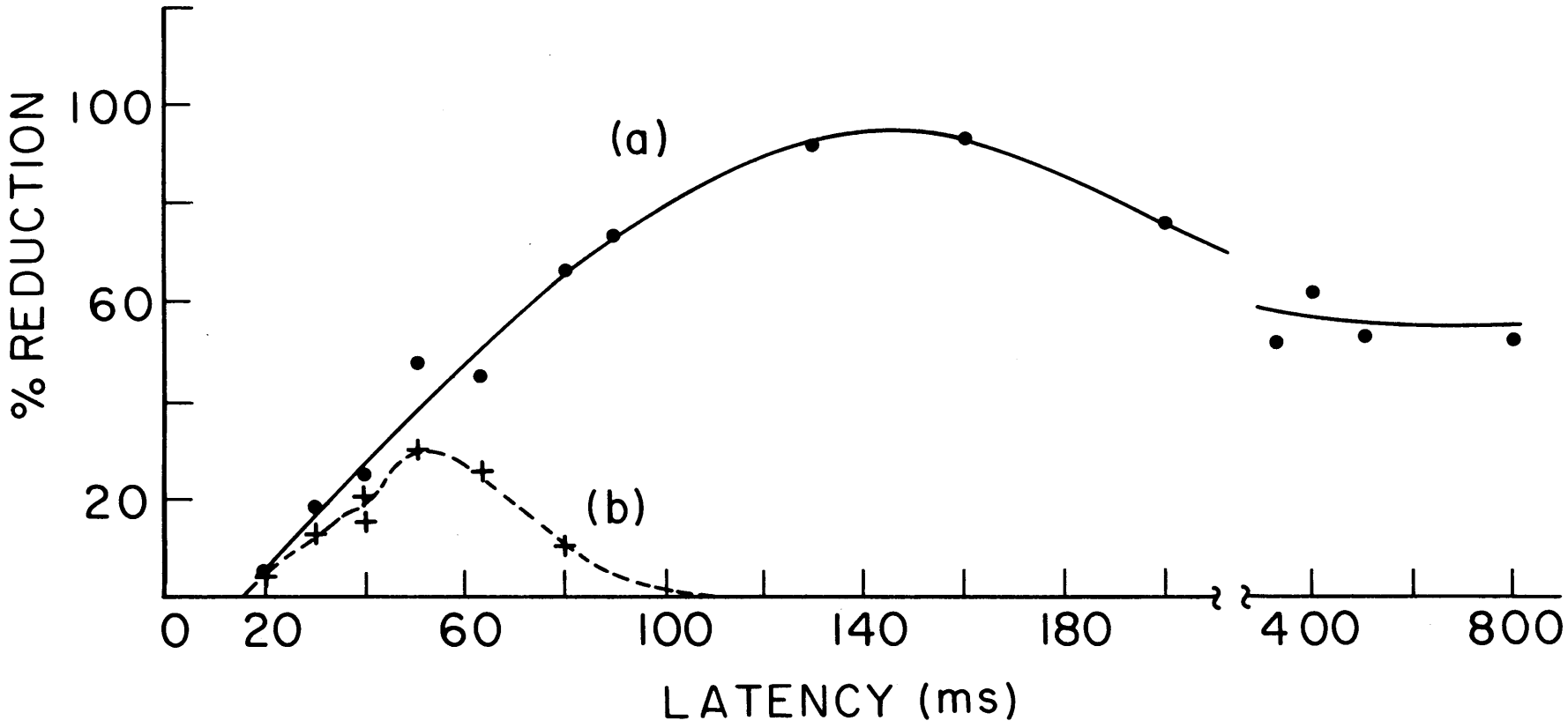
This is part of the series illustrated in Fig. 11, in which the peripherally evoked DRP was preceded by conditioning PT trains of varying lengths.

PT stimulus train, shown by dark bar at bottom, was 1.6 volts (2 times flexion threshold). Vertical scale for DRP's; 100 μV./div.; horizontal scale: 50 ms./div.

Figure 11. Time course of reduction of a peripherally evoked DRP by continuous PT stimulation.

(a) plots the reduction of a peripherally evoked DRP by a preceding PT train. "Latency" means the time between the first PT shock and the peak of the peripherally evoked DRP; during this time the PT was continuously stimulated. PT stimulation was usually terminated after the peripherally evoked DRP, as in Fig. 10, which gives an example from this series. The result is essentially the same as if a continuous PT train were initiated and the peripheral DRP evoked at various latencies after the first PT shock. The reduction was computed according to formula II-3. In this experiment the reduction was unusually large, but exhibited the same time course as that seen in other experiments. The peripherally evoked DRP was produced by a single shock to the skin.

(b) shows the DRP evoked by a long PT train. This experiment demonstrates that long PT trains may reduce the effectiveness of afferent volleys in producing a DRP even after the PT-DRP returns to zero.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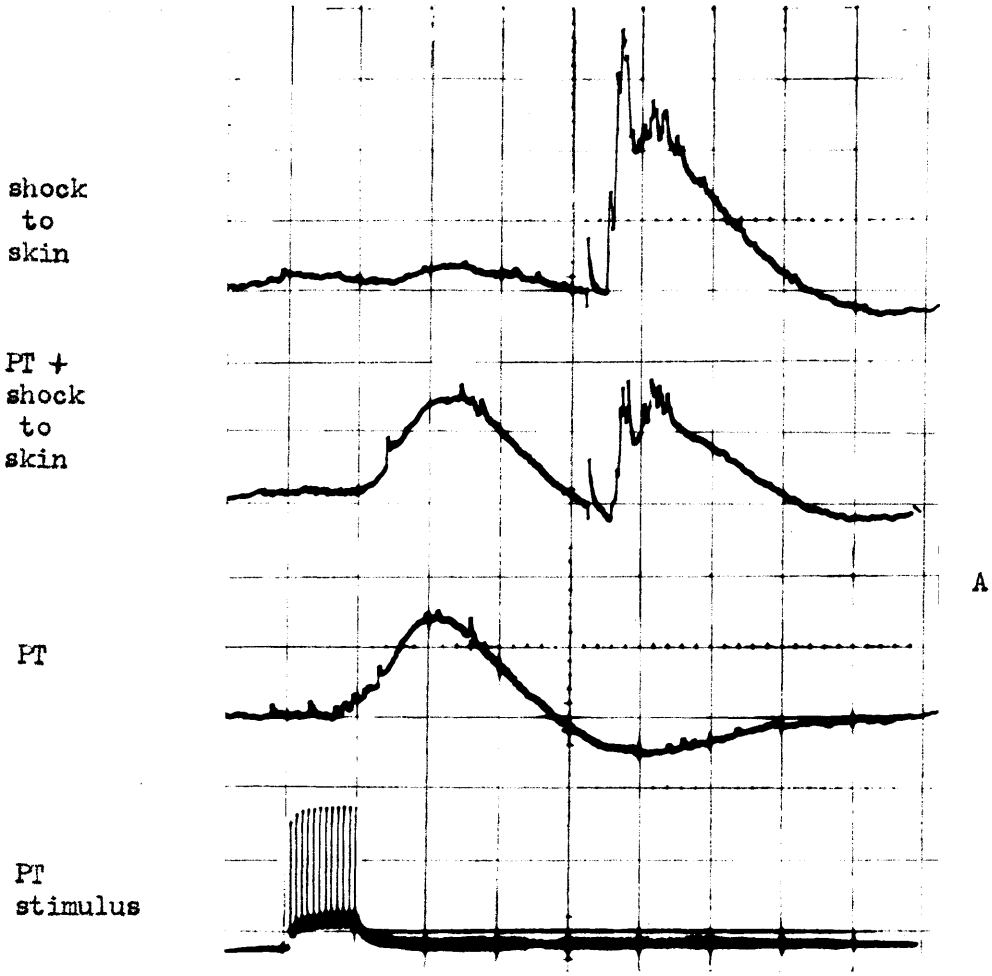
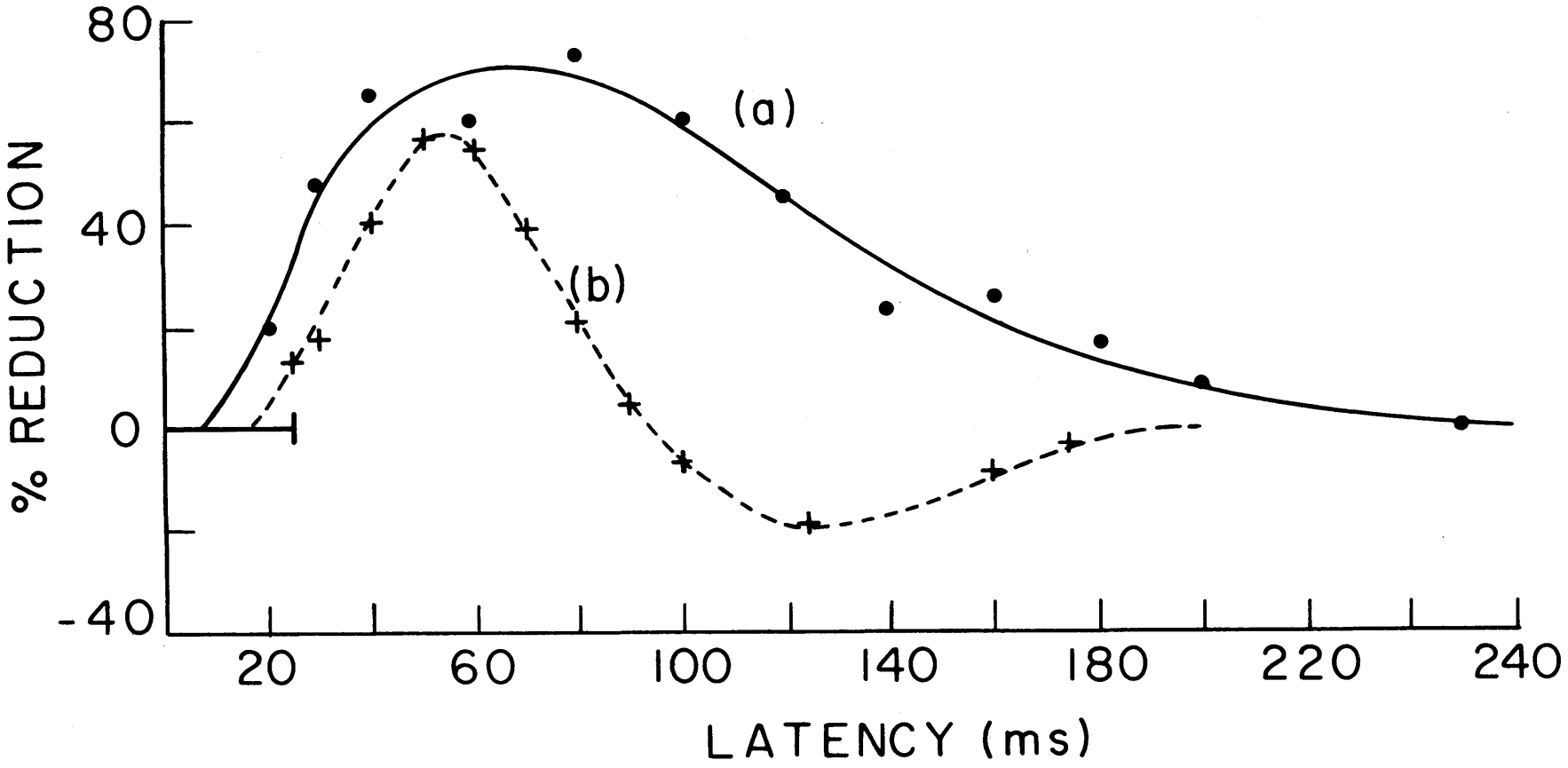


Figure 12. Reduction of peripherally evoked DRP by a short conditioning PT train.

Part A (above) shows DRP's evoked by PT and peripheral stimuli as indicated. The interesting feature is a reduction of the peripherally evoked DRP during the positive phase of the PT-DRP. If the afferent fibers were depolarized during this phase, the peripherally evoked DRP would have been enhanced instead of reduced. Vertical scale for DRP's: 200 μV./div.; horizontal scale: 25 ms./div.

Part B (next page) gives the time course of reduction of the first peak of the DRP, i.e., the dorsal root reflex, as computed by formula II-3. (a) plots reduction as a function of latency of the dorsal root reflex after the first PT shock. (b) plots the PT-DRP for comparison. The line from 0 to 25 ms. indicates duration of the PT stimulus train.


80
40
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% REDUCTION
(a)
(b)
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220
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LATENCY (ms)

still substantially reduced even when the PT-DRP exhibited a positive phase.

III. Single Interneuron Responses.

A. Criteria for characterizing cells.

Single cells recorded in the dorsal horn were characterized by the following criteria:

Depth below the cord surface was measured by the micrometer drive, but due to the considerable variability of cord geometry and zero position at the dorsum this gave a relatively unreliable estimate of the location of cells in the dorsal horn. More useful was the relative depth of successive units encountered in advancing the electrode. The position at which we encountered numerous small spike responses to brushing the skin was taken to indicate the dorsal border of lamina 3; the relative depth of cells below this level was useful in ordering them into layers. For most tracks the electrode was cut off and a transverse section of the cord containing the electrode fixed and examined. This gave a more accurate estimate of the depth and lateral position of cells in the dorsal horn.

Modality of effective natural stimulation and receptive field were important criteria in defining the layer of the cell. A cell was categorized as a layer 4 unit if it responded to skin stimulation over a small receptive field. At

rostral L7 neurons in the medial part of lamina 4 had receptive fields covering one of leading toes or less. Lateral cells had receptive fields on lateral toes and the lateral side of the foot. Receptive fields on the side of the foot were generally larger than those on the toes. These cells responded briskly to brushing the skin and often increased their response to touch and pressure. If maintained pressure produced a more intense, sustained response than brushing, the cell was characterized as having a wide dynamic range; cells showing no increase, or a decrease in firing to pressure were said to have a small dynamic range. Layer 5 cells also responded only to skin stimulation, but had larger receptive fields than layer 4 cells. These fields usually included several toes or large areas of the foot and leg. A sensitivity gradient was a common characteristic of these fields; the threshold to natural and electric stimulation often increased toward the periphery of the receptive field. Inhibitory regions could also be found for some cells. Layer 6 cells generally responded to both cutaneous and proprioceptive stimulation of the ipsilateral limb. Cutaneous receptive fields usually resembled those of layer 5, but more often exhibited an inhibitory region. In spinal (guillotined) animals the responses to joint movement were more often absent than in decerebrate preparations. Any cell responding to contalateral stimulation was assumed to belong to layer 7; these deeper cells generally also exhibited more

delayed and variable responses to any stimulation.

Minimum latency with respect to electrical stimulation of the skin and/or the dorsal root was measured. Although with peripheral stimulation there was an average increase in latency of about 1.5 ms. between layers 4 and 6, this was a statistical effect, and not useful for separating individual units into layers. Dorsal root stimulation gave no noticeable difference in latency with layers. This differs from the result of Wall, (1966) who found 2 ms. increases of latency between successive layers, but using threshold intensities.

Spontaneous activity was characterized as to five degrees of intensity and nature (regular, bursting). Layer 6 cells were found more likely to exhibit regular spontaneous firing.

In some experiments the dorsolateral column (DLC) was stimulated at L3 or L4 to determine whether the axon of a cell ascended in this tract. Criteria for antidromic response were an all-or-nothing spike response at a short (.5-1.5 ms.) invariant latency, and response to stimulus pairs separated by 1.3 ms. or less. The difference between antidromic stimulation via the DLC and orthodromic stimulation via the dorsal columns was easily discernible. In some experiments recordings were made from axons in the DLC at the L5 level -- presumably below the level at which axons from Clarke's column join the tract.

PT influences on these cells were investigated by stimulating the PT with 0.4 ms. pulses at 500/sec., either con-

tinuously or with brief trains of 50 ms. or more. Responses of single units generally varied the same way as behavior or slow potentials, with variations in PT parameters (Fig. 4c). PT stimulus intensities were usually kept below three times flexion threshold. We observed the effects of PT stimulation on the cell's spontaneous activity and its responses to natural stimulation and to electric shocks applied to the skin. Generally the PT had the same effect on all three types of activity -- i.e., if the PT reduced spontaneous activity it usually also reduced the response to peripheral stimulation. However, the thresholds at which the PT began to influence the cell noticeably were not always the same for the three types of activity. Often a cell's response to electrical stimulation of the skin could be reduced by a preceding 50 ms. PT train at lower intensities than those necessary to noticeably inhibit spontaneous activity with continuous PT stimulation. Some cells showed a mixed response -- excitation followed by inhibition or vice versa; these are discussed below.

B. Cell Responses to PT stimulation.

1. Layer 4

Of the 64 cells characterized as layer 4 units, almost half (26) were unaffected by PT stimulation and half (27) were inhibited. When a cell was unaffected we always veri-

fied that PT effects could still be shown on slow potentials or other single cells. The inhibition generally began about 15-20 ms. after the start of PT stimulation and continued to 50-100 ms. beyond the end. However, with prolonged PT stimulation the effect began to wane after about 5-10 seconds; whether this effect was physiological or artifactual could not be decided, and stimuli of many seconds' duration were generally avoided. The average threshold intensity at which inhibition could be discerned was about 1.4 times flexion threshold. Fig. 13 illustrates inhibition of activity by a 50 ms. PT train.

A few (9) layer 4 cells showed mixed excitation and inhibition. Of these about half fired during the major portion of the PT stimulation and were inhibited for a subsequent period. Some evidence was obtained that barbiturates abolished the excitation but not the inhibition. The other half of the mixed group responded weakly at latencies between 18 and 22 ms. and were inhibited for the remainder of the PT stimulation. Fig. 14 illustrates such a unit, with the accompanying DRP.

Fig. 15 illustrates a useful method of demonstrating PT inhibition. An electric shock was given in the receptive field, first alone and then with a preceding 50 ms. PT train. The reduction of the cell response is seen to correlate with a reduction of the DRP.

A weak correlation was found between dynamic range and PT influence (Table I). A larger percentage of the wide

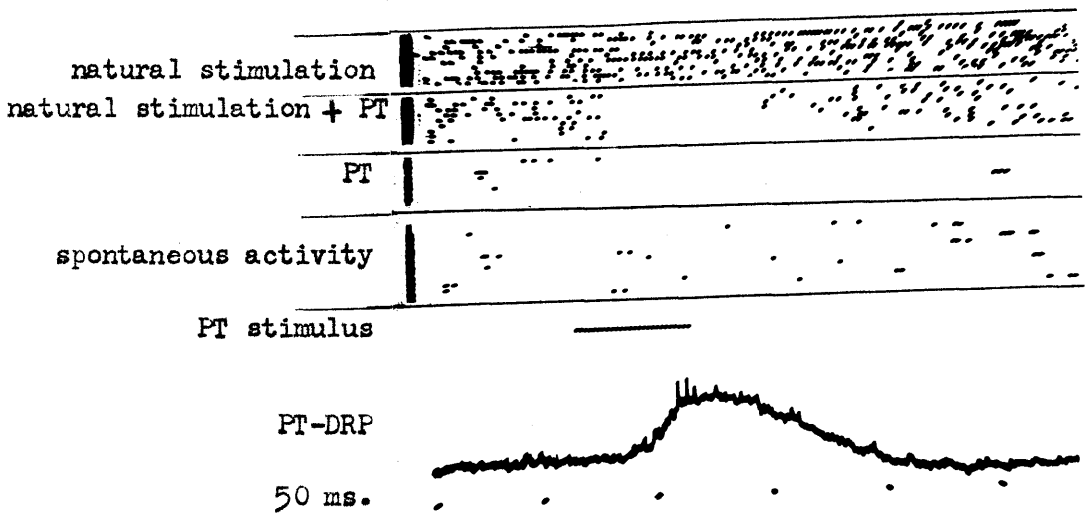


Figure 13. Layer 4 cell inhibited by PT.

Top half is the raster display of cell responses. A sequence of cell firings was converted to a horizontal row of dots, and successive sequences displayed in adjacent rows from top to bottom. Here, the duration of one horizontal sweep was 300 ms. and sweeps were repeated every second (the responses occurring in the 700 ms. interval between sweeps are displayed at the same position at the extreme left).

"natural stimulation": responses of cell to brushing skin of receptive field (10 repetitions are shown).

"natural stimulation + PT": responses of cell when skin was brushed and PT stimulated for 50 ms. as shown below.

"PT": responses when 50 ms. PT train was given alone.

"spontaneous activity": activity in absence of stimuli.

"PT stimulus": duration of PT stimulus train (50 ms.).

"PT-DRP": dorsal root potential evoked by 50 ms. PT train alone.

"50 ms.": time markers, spaced 50 ms. apart.

Note that PT stimulation inhibits the response of the cell to brushing the skin; the inhibition seems to coincide with the major portion of the DRP.

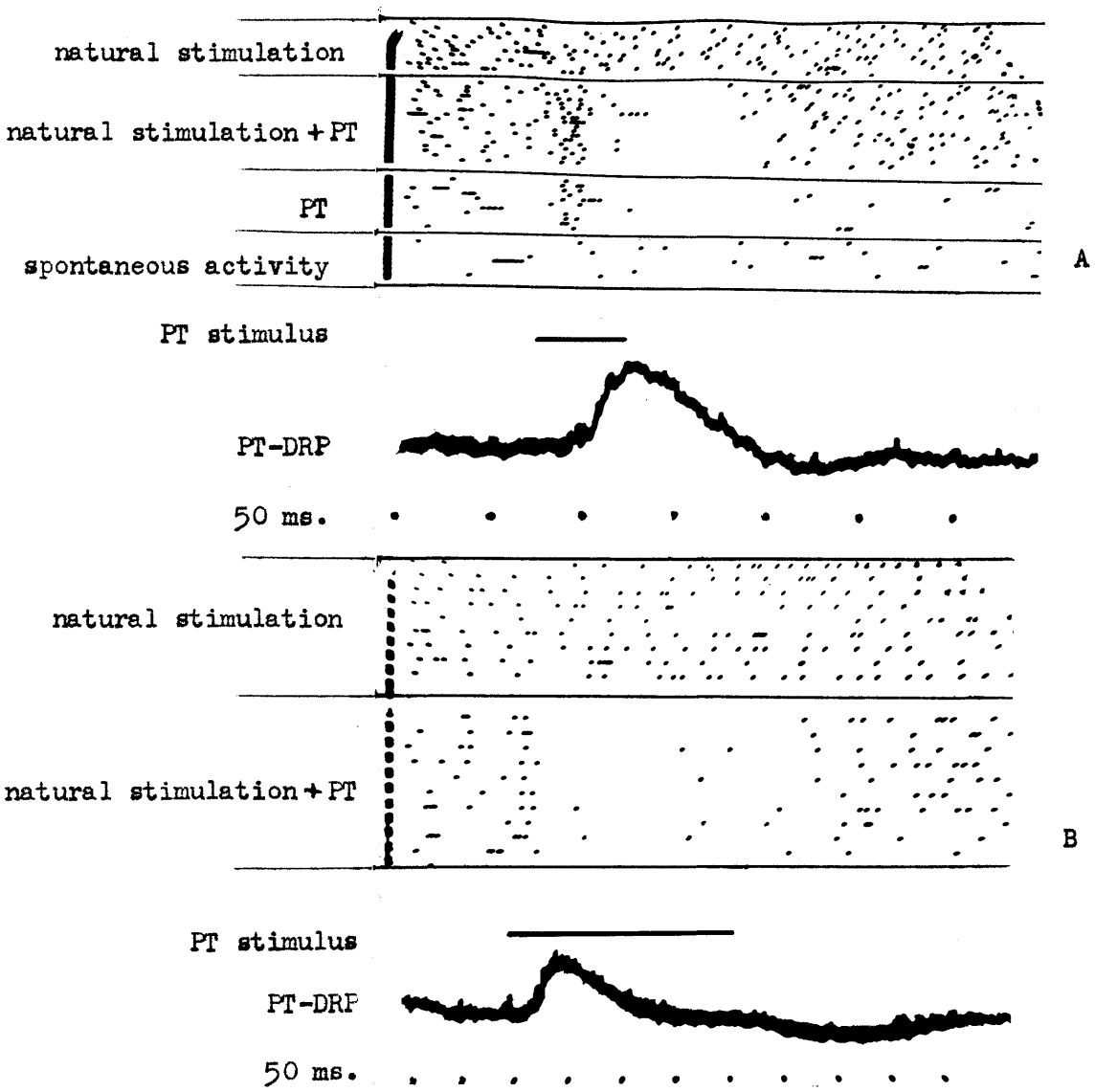


Figure 14. Layer 4 cell showing mixed excitation and inhibition by PT.

Note different time scales for parts A and B. Duration of the PT train was 50 ms. in part A and 200 ms. in part B. This cell showed a brief period of firing preceding a longer inhibition. In part B, inhibition was maintained for the duration of the PT train, although the DRP returned to zero.

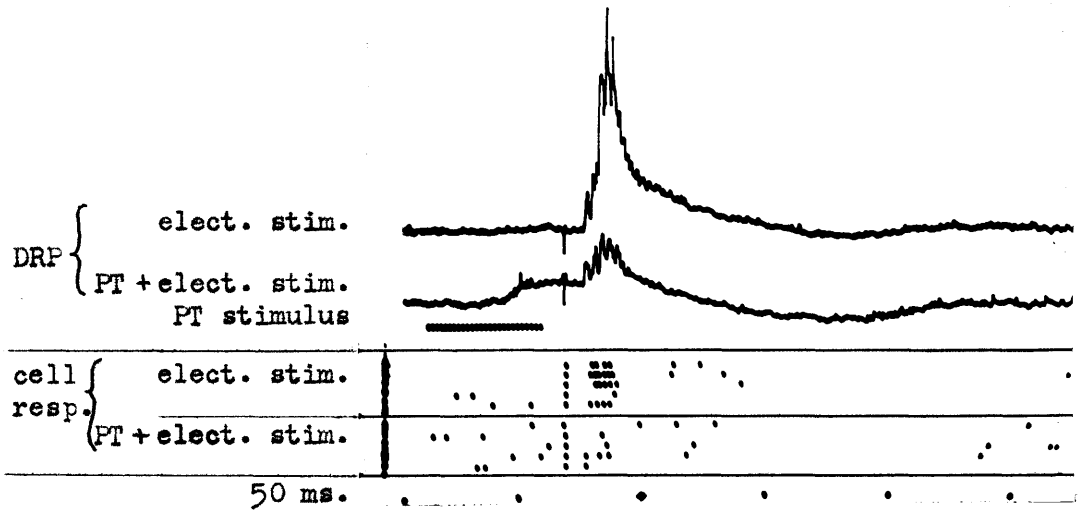


Figure 15. PT inhibition of cell response and DRP.

This figure shows the simultaneous reduction of a peripherally evoked DRP and the firing of a DLC layer 4 cell. Both effects reflect a decreased postsynaptic effectiveness of the afferent volley, supporting the possibility of presynaptic inhibition by the PT.

"elect. stim." labels the responses evoked by a single shock to the skin. "PT + elect. stim." labels the responses when the shock was preceded by a 50 ms. PT train.

The cell response is the row of dots following the stimulus artifact dot. This cell was recorded in the dorsolateral column; its receptive field was a fraction of one toe, typical of layer 4 cells (but since its cell body was not localized it was not included in the layer 4 population).

This figure illustrates a commonly used test for inhibition by the PT, namely the reduction of response to a peripheral shock by a preceding 50 ms. PT train.

TABLE I

Effects of PT on cells grouped by dynamic range.

LAYER	DYNAMIC RANGE	PT EFFECTS 0	—	+/—	+	% AFFECTED BY PT (—, + or +/—)
4	small	8	3	1	0	33%
	wide	11	15	3	1	63%
5	small	2	1	0	2	60%
	wide	4	8	9	7	86%

dynamic range cells exhibited inhibitory influences via the PT as compared to medium or small dynamic range units.

2. Layer 5.

The 58 cells characterized as layer 5 units were about equally divided among the four possible categories of PT influence: 13 unaffected, 15 inhibited, 17 excited and 13 with mixed effects.

The inhibition resembled that seen in layer 4 with respect to time course; its average threshold was also 1.4 times flexion threshold.

Cells showing mixed effects behaved differently than their layer 4 counterparts. Generally their firing period began at 15-20 ms. latency and ended between 30 and 40 ms., to be followed by an inhibitory period. Thresholds at which the excitation and inhibition could be discerned both averaged to 1.7 times flexion threshold.

Those layer 5 cells excited by the PT began firing about 15-20 ms. after the beginning of PT stimulation and continued up to 50 ms. after the end. Fig. 16 illustrates a layer 5 cell which exhibited pure excitation; no inhibition of response to peripheral stimulation was seen. The average threshold at which these cells began firing was about 1.3 times flexion threshold. Excitation of a cell by the PT could be inhibited by stimulating its inhibitory receptive field, if it had one.

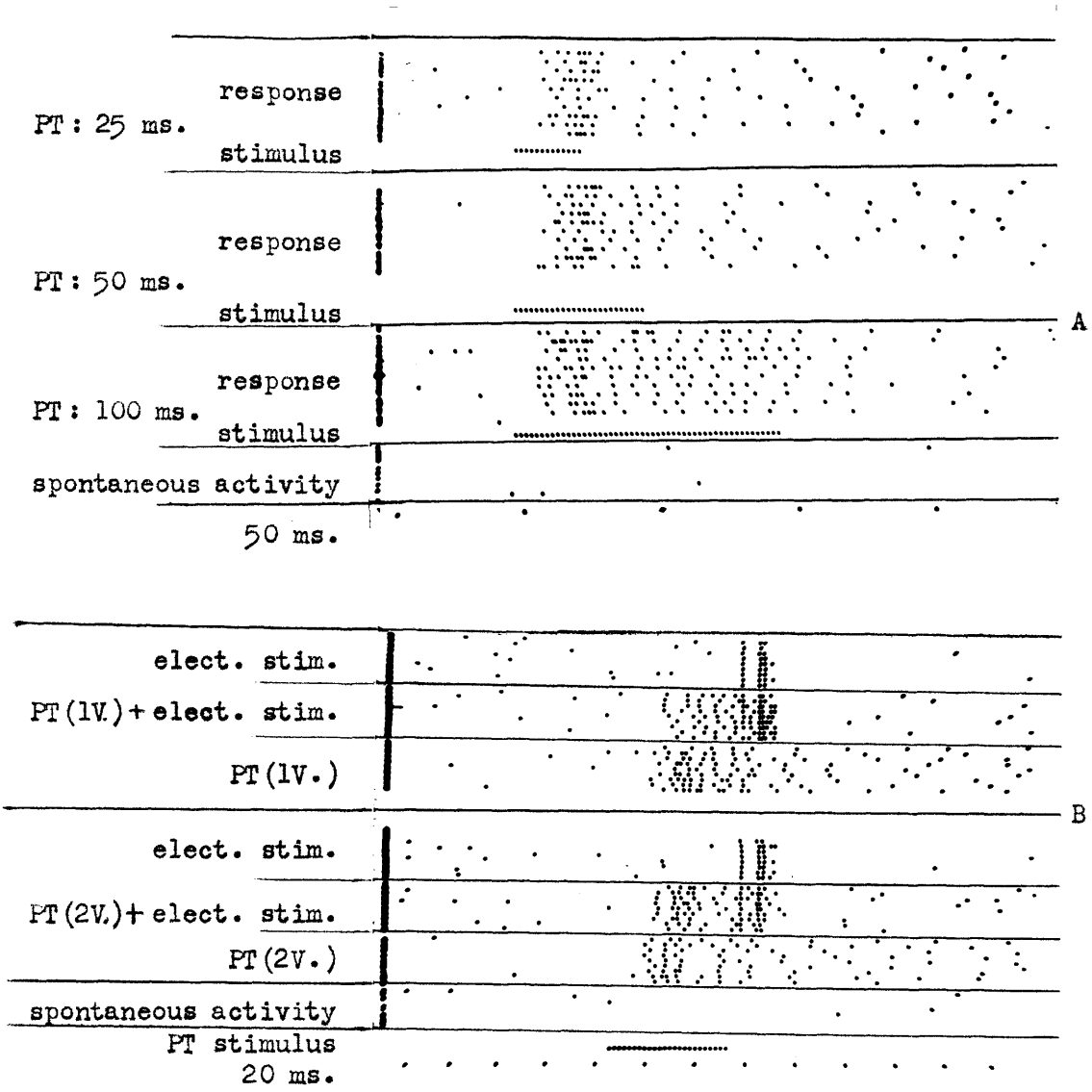


Figure 16. Layer 5 cell excited by PT.

Part A: responses of the cell to different lengths of PT stimulation, as indicated. PT stimulus intensity was 2 volts.

Part B: effect of a 50 ms. PT train on the response to an electric shock in the center of the receptive field. Repeated at two intensities of PT stimulation: 1 volt and 2 volts.

This cell showed no signs of inhibition by the PT. With a 1 volt conditioning PT train, the response to peripheral shock was even enhanced, at a time after the PT train when other cells show maximum inhibition.

As with layer 4, the wider dynamic range cells of layer 5 had a larger proportion inhibited by the PT. In addition a slightly larger percentage of layer 5 cells (85%) had wide dynamic ranges as compared with layer 4 cells (71%).

3. Layer 6

Of the 45 cells characterized as layer 6, the majority (32) were excited by the PT. Relatively few showed inhibition (4) or no effects (3) or mixed effects (6). Fig. 17 illustrates one of the inhibited layer 6 cells.

The cells which were excited generally began firing 15-20 ms. after the beginning of PT stimulation and continued up to 15-20 ms. beyond the end (Fig. 18). A few cells began responding at somewhat longer latencies (Fig. 19). Prolonged PT stimulation over several seconds produced sustained firing in some cells and periodic bursts of activity in others. The firing pattern of this latter group exhibited the same periodicity as the alternate flexion and relaxation of the hindlimb elicited by continuous PT stimulation in unparalyzed preparations. The average threshold for exciting a layer 6 cell was 1.2 times flexion threshold.

In spinal preparations over half the cells which were characterized by depth as layer 6 cells had no demonstrable proprioceptive input. In decerebrate animals the proportion of layer 6 cells responding to joint movement was larger, but this proportion could not be estimated from our data

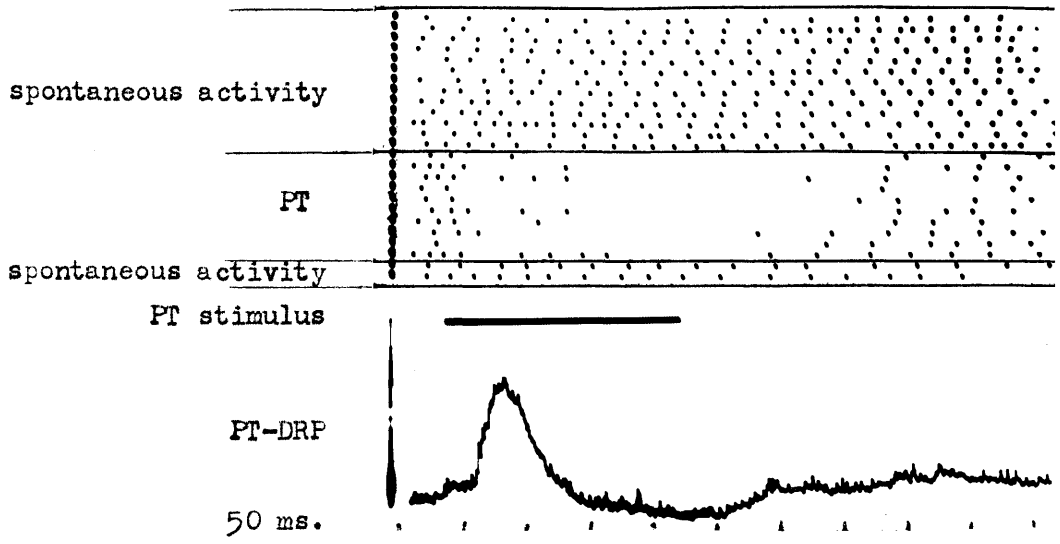


Figure 17. Layer 6 cell inhibited by the PT.

This figure illustrates inhibition of spontaneous activity by a 200 ms. PT train at 5 volts. There is no evidence of an off response at the termination of the PT stimulus.

This unit had both excitatory and inhibitory cutaneous receptive fields, and responded to flexion of ankle and extention of toes.

The regular spontaneous activity exhibited by this unit is typical of layer 6 cells.

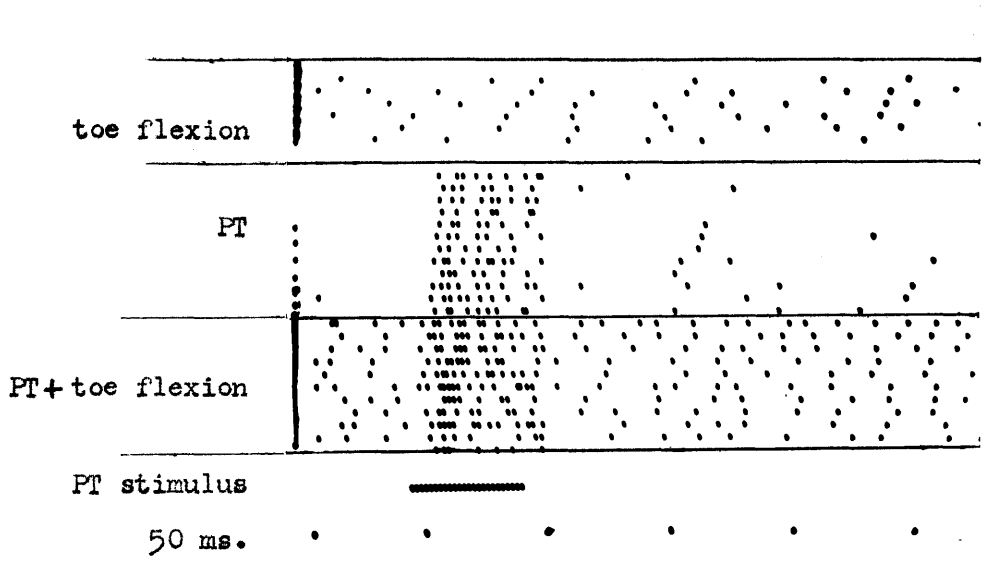


Figure 18. Layer 6 cell excited by the PT.

This cell responded to tonic flexion of toes, as shown. Excitation by PT and flexion are additive; there is little evidence of inhibition of responses following the excitatory period.

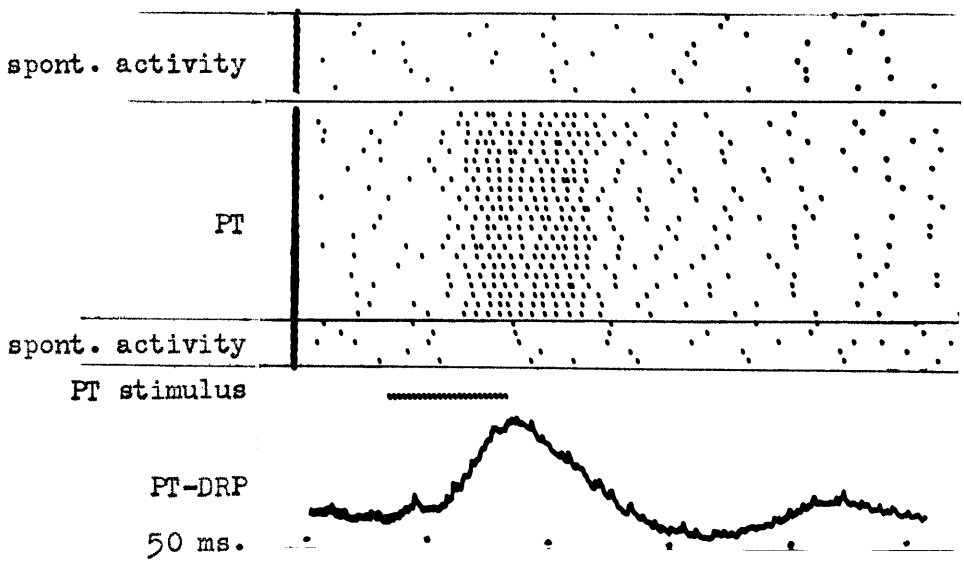


Figure 19. Layer 6 cell excited by PT after delay.

Some layer 6 cells responded to PT stimulation at relatively long latencies. As shown, the firing of this cell was synchronous with the PT-DRP.

because many purely cutaneous cells were rejected in a search for proprioceptive units. The cutaneous and proprioceptive layer 6 cells did not differ with respect to PT influence -- i.e., the fraction of each group exhibiting a given type of PT influence was about the same. Thus the "cutaneous" layer 6 units differed from the layer 5 cells in having a much greater proportion excited by the PT.

Of 21 cells whose response to joint movement could be clearly characterized, 14 responded to passive flexion, 5 to extention, and 2 to extention of one joint and flexion of another. The flexion-responsive cells did not differ from those responding to extention with respect to PT influences.

4. Dorsolateral Column.

50 cells whose axons ascended in the DLC were characterized with respect to PT influences. These units were obtained in two ways: (a) 25 cells were recorded in the dorsal horn and identified as DLC units by antidromic responses, and (b) 25 cells were recorded in the DLC and verified to respond to cutaneous stimulation. PT influences on these two groups were the same. The units in group (a) were also included in the layer populations; those of group (b) were not, since their cell bodies were not located.

An interesting finding of this study was that the dorsal horn cells sending axons up the DLC were more likely to be inhibited by the PT than those that did not. Of the 50

DLC cells, 31 were inhibited, 9 unaffected, 9 exhibited mixed effects and 1 was excited. The inhibition had the same time course and threshold as that described for layer 4 and 5 cells, and is illustrated in Fig. 13. All three types of mixed excitation and inhibition described for layers 4 and 5 were seen in the DLC units.

A question of obvious interest is where do the DLC axons originate? This can be answered by locating the cell bodies in the dorsal horn which respond to antidromic firing of the DLC, as was done for group (a). Of these 25 cells, 19 (76%) were characterized as layer 4, and 6 (24%) as layer 5. To gather more statistics, an additional experiment was done in which the cell bodies of 18 antidromically fired DLC axons were located by depth, but no characterization of responses attempted. In this group 83% of the cell bodies were in layers 3 or 4, 17% in layer 5.

A slightly different question, also answered by these studies, was what fraction of cells of any layer send axons up the DLC? Of the 44 layer 4 cells tested, 19 (43%) could be fired antidromically from the DLC. Of 34 layer 5 cells tested, 6 (18%) were DLC units. None of the 14 layer 6 cells tested gave antidromic responses from the DLC.

When the sural nerve was stimulated electrically, the DLC units gave the responses described by Wall (1959) and Mendell (1965): an initial burst of rapid firing evoked by the afferent volley in the large A fibers, followed by a silent period, and then the late bursts of firing produced

by volleys in the C fibers. At sufficiently high intensities the PT could abolish both responses (Fig. 20). At lower PT intensities, responses to both A and C volleys were inhibited, but sustained PT stimulation gradually became less effective after several seconds. The resumption of C responses during continuous PT stimulation follows the "windup" pattern described by Mendell and Wall (1965). A brief PT train abolished part of the C responses and left subsequent ones intact (Fig. 21).

5. General observations.

The PT effects on the various groups of single cells is summarized in Fig. 22. For the layer populations the units observed in spinal preparations are distinguished from those seen in decerebrate cats. As shown, the PT effects were the same in both preparations. Similar observations were made in mapping the PT-evoked field potentials before and after guillotining; there was no change in the field potential distribution after the cat was guillotined.

Cells recorded in the medial third of each layer were compared with those recorded in the lateral third. No statistically significant difference could be found between the medial and lateral division of any layer.

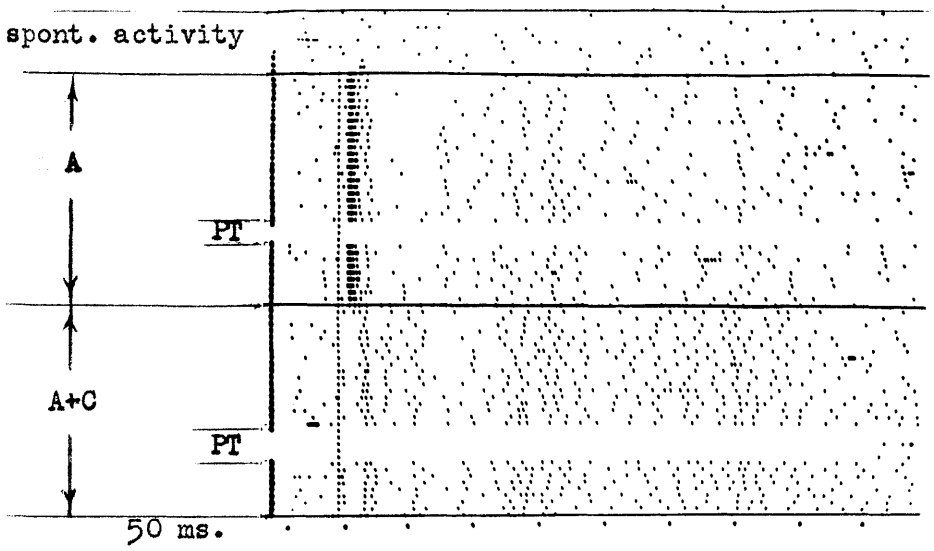


Figure 20. PT inhibition of DLC cell responses to volleys in sural A and C fibers.

In this experiment the sural nerve was dissected and stimulated electrically. Cell responses were recorded in the DLC.

During "A" the stimulus was of sufficient strength to excite the large A fibers, The DLC cell responded with a typical burst of activity, shown here following the stimulus artifact. Continuous PT stimulation was applied during the interval marked, and abolished all cell responses.

During "A + C" the sural nerve was stimulated at a strength sufficient to excite the lower threshold C fibers, as indicated by the appearance of a C fiber compound action potential recorded from the nerve. The cell responded with typical late bursts of activity evoked by the afferent C fiber volleys. At this strength the response to A fibers was curiously reduced, possibly because A fibers were blocked by stimulus spread. As shown, continuous PT stimulation abolished the responses to C fibers.

PT intensity was 4 volts (4 times flexion threshold). With lower PT intensities both A and C fiber volleys were partially reduced.

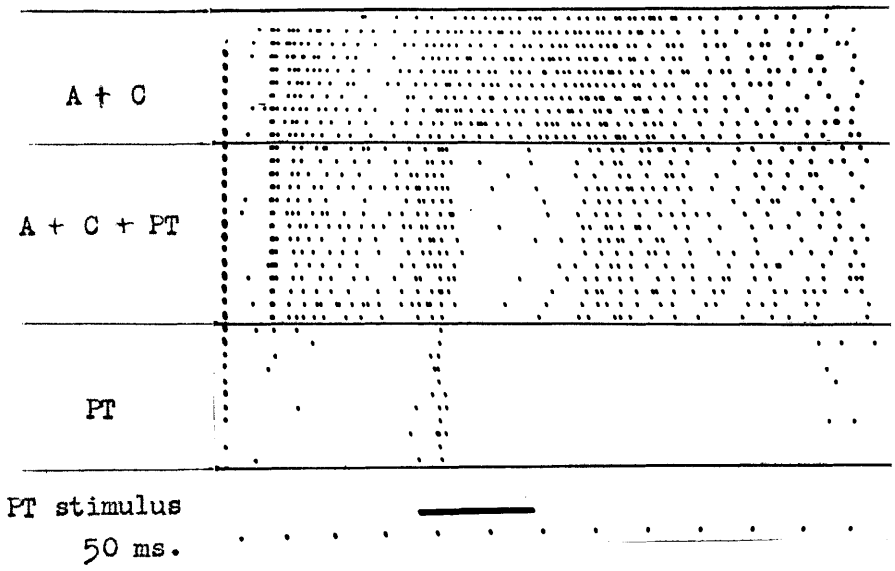


Figure 21. Brief PT inhibition of DLC cell response to C fibers.

Electrical stimulation of the sural nerve at strengths sufficient to stimulate C fibers produced the late responses shown in "A + C".

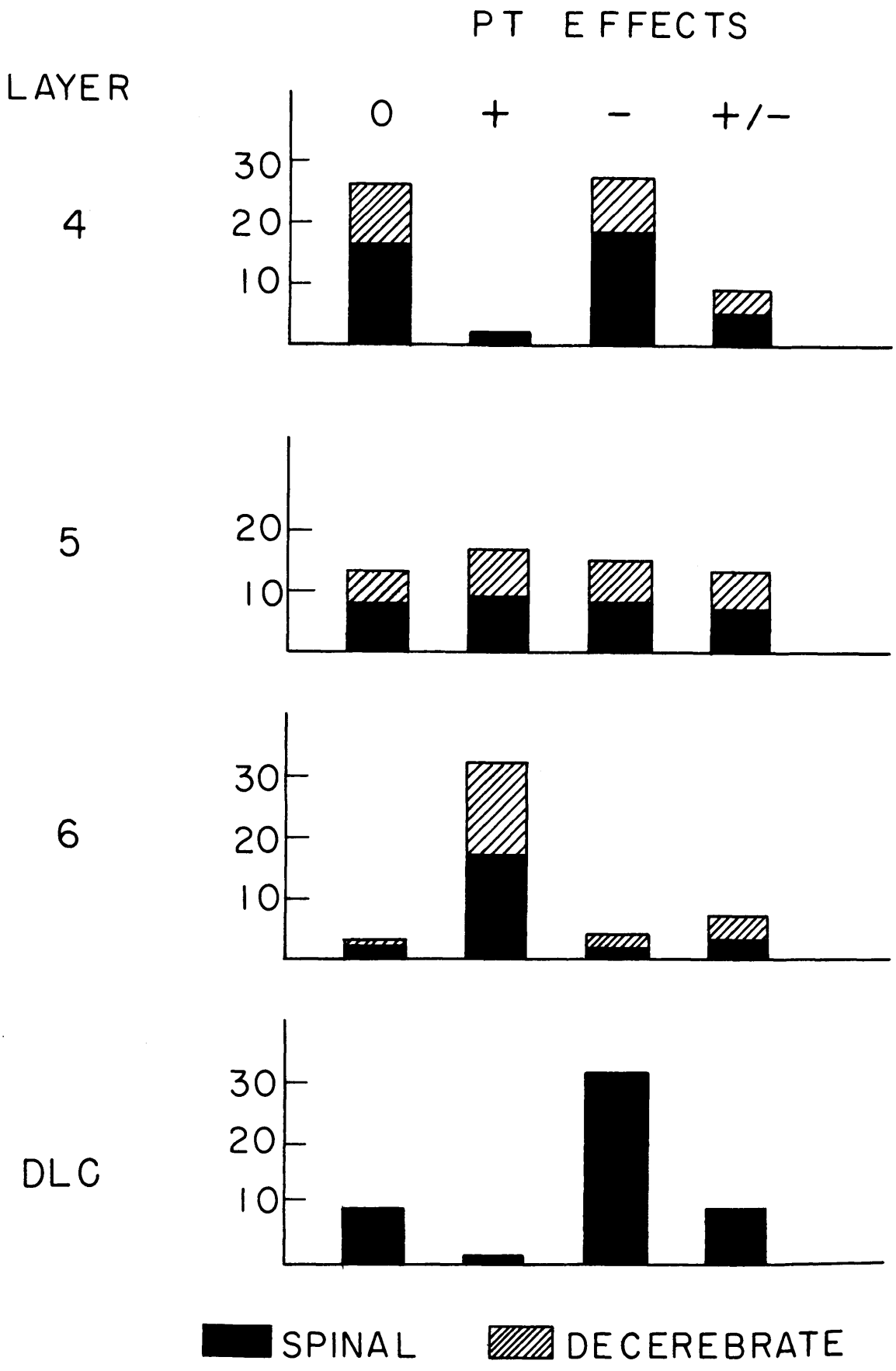
A 100 ms. PT train abolished a portion of the responses to C fiber volleys, leaving subsequent responses intact ("A + C + PT").

The PT stimulus alone produced a brief firing of the cell ("PT").

Figure 22. PT effects on dorsal horn interneurons.

This summary diagram shows the effects of the PT on cells of different layers. The units recorded in decerebrate cats are distinguished from those recorded in guillotined cats; the PT effects were the same for each preparation.

The cells with axons in the dorsolateral column are shown separately (no distiction between decerbrate and guillotined cats was made here). This population includes cells recorded in layers 4 and 5, with antidromic responses to DLC stimulation, and cells recorded in the DLC with cutaneous responses. These two sub-groups did not differ with respect to PT effects.


PT EFFECTS
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SPINAL
DECEREBRATE

DISCUSSION

I. Responses to PT stimulation.

Stimulating the medullary pyramids of "guillotined" or decerbrate cats was observed to evoke the following types of responses: (a) flexion of the contralateral hind limb, (b) field potentials in the lumbar spinal cord, (c) dorsal root potentials in lumbar dorsal roots, and (d) changes in the activity of single neurons of the lumbar dorsal horn. All these responses were found to vary together when the PT was stimulated with various parameters (Fig. 4). The optimum stimulus parameters were trains of 0.4 ms. square pulses at 500/sec.

Stimulation with 0.1 ms. pulses at 100/sec. was found to be among the least effective; this may help explain why Wall (personal communication), using these parameters, was unable to demonstrate inhibition of layer 4 cells. Also, inhibition could often be more clearly demonstrated by the effect of a short PT train (50 ms. or less) on responses to peripheral stimulation, than by a continuous PT train on spontaneous activity.

II. Source density mapping of PT-evoked field potentials.

The field potentials evoked by PT stimulation were used in a preliminary study to locate the interneurons excited by

the PT. A method of analyzing these potentials to derive their sources was described. Applying this method, the source density was found to be greatest in layers 5 and 6. The fact that subsequent single cell recordings demonstrated the active cells to be confined to this region supports the assumptions on which the method is based. Using just the height of the field potentials as an index of activity would have led to the conclusion that cells throughout the dorsal horn were excited by the PT. The expedient of delimiting just regions of largest field potentials as regions of activity (Coombs, Curtis and Landgren, 1956) does not define the boundary of these regions accurately. Defining the limits as some percentage of the maximum height is a variable and arbitrary procedure at best. As demonstrated here, the method of locating the source density defines boundaries which are in excellent agreement with the limits of activated cell populations.

It should be pointed out that a new and direct derivation of the formula for the source density of field potentials was presented. The final expression (II-3) is identical to the one derived by Howland, et. al. (1955) and Kornacker (1962) on the basis of a specific but more elaborate model. Their model related the only measurable variables -- potential and position -- to several unmeasured variables -- currents, resistance, neural activity -- and in the process invoked some assumptions of dubious validity. Specifically, these assumptions include: (a) the extracellular currents pro-

duced by active neurons are the sole generators of the field potentials, (b) the difference in potential recorded at two points is due only to the current flowing through the resistive medium, (c) Ohm's law is valid in regions containing current sources, (d) spatial and temporal variations in conductivity can be ignored, and (e) the medium is non-polarizable. As shown by our more direct derivation, these questionable assumptions are not necessary to preserve the point of the method, namely to locate the sources of the field potentials. These "sources" need not be identified with one specific physical phenomenon, such as currents or dipoles; indeed the complexity of the actual physiological situation would preclude such a simple identification. All that is required is the accurate localization of the neural events causing the field potentials, whatever they be. The simple approach proposed here is based on two assumptions: (a) the expression giving the source density of the field potentials is the common expression for the source of any scalar physical field -- viz., the Laplacian of the potential, and (b) the source density so defined is proportional to the number of active neurons. No additional unmeasured variables need be involved; the relation of the potentials to specific physical events, such as current flow or polarization, is left undefined until such phenomena become experimentally distinguishable.

III. Dorsal Root potentials and presynaptic inhibition.

Considerable evidence indicates that a negative dorsal root potential is correlated with a depolarization of the afferent dorsal root fibers. The excitability of the afferent terminals, as tested by their threshold to extracellular stimulation (Wall, 1958), increases in parallel with the DRP. The membrane potential recorded intra-axonally in large afferent fibers exhibits a depolarization which follows the DRP (Eccles, Schmidt, and Willis, 1963).

Correlated with this depolarization is a decrease in the postsynaptic effectiveness of afferent volleys -- a phenomenon called "presynaptic inhibition". The postsynaptic effectiveness is often measured by the response of the postsynatpic cells; when the reduction of such a response follows the time course of a simultaneous negative DRP, it is often concluded that the inhibition is presynatpic (Eccles, 1964). Strictly speaking, this inhibition must be shown to be independent of any change in membrane potential or excitability of the postsynaptic cell before it can be called presynaptic (Frank and Fuortes, 1957). However, the difference in time course of pre- and postsynaptic inhibition is often used to distinguish them.

The depolarization of the afferent fibers is thought to be generated by the activity of special interneurons which synapse on these fibers, close to their termination in the cord. The location of these interneurons is still disputed.

Wall (1962, 1964) argues that the small cells of layers 2 and 3 are ideally located and interconnected to perform this function. Eccles, Kostyuk and Schmidt (1962) found cells in the ventral part of the dorsal horn which were excited during the rising phase of the DRP and identified these "D cells" as the generators. A new possibility for settling this issue is discussed below.

Mendell and Wall (1964) have recently shown that volleys in small cutaneous fibers can produce a positive DRP, which is correlated with a hyperpolarization of the afferent terminals, as demonstrated by a decrease of their excitability. This hyperpolarization was postulated to correlate with an increase of postsynaptic effectiveness of any afferent volleys -- i.e., a presynaptic facilitation. They demonstrated that a tetanus of the small C-fibers, which produced no ventral root reflex itself, could facilitate such a reflex evoked by a volley in the large A fibers. (However, since this reflex is polysynaptic, such evidence for presynaptic facilitation remains indirect.) Mendell and Wall also showed that the DRP evoked by a mixed volley in the large and small fibers had separable negative and positive components; the positive DRP could be enhanced and the negative occluded by superimposing a tonic negative DRP produced by a tetanus of an adjacent dorsal root.

We found that the DRP evoked by a short PT train generally had a large negative phase followed by a smaller positive phase. When such a PT-evoked DRP was superimposed on

the tonic negative DRP evoked by continuous touch of the skin, the negative phase was reduced, but there was no enhancement of the positive phase (Fig. 9). This indicates that the positive phase probably does not reflect the type of hyperpolarization seen by Mendell and Wall with peripheral nerve stimulation.

This is further supported by the evidence of Fig. 12. A peripherally evoked DRP was timed to coincide with the positive phase of the PT-DRP. If the afferent fibers had been hyperpolarized during this phase, the peripheral DRP should have been augmented instead of reduced. Thus, our evidence indicates that the PT exerts only a depolarizing effect on the afferent fibers. The positive phase of the PT-DRP may be due to other activity in the cord which is picked up by the dorsal root acting as a wick electrode.

The negative phase of the PT-DRP was more clearly correlated with a decrease in postsynatpic effectiveness of an afferent volley, as measured either by the size of the DRP evoked by the volley or by the response of layer 4 cells to the volley (Fig. 15). However, as discussed in the next section, not all afferent fibers seemed to be equally affected.

IV. PT effects on single dorsal horn interneurons.

The effects of the PT on the cells of the dorsal horn was found to be different for each layer. (Fig. 22). About

half the layer 4 cells were inhibited and half unaffected. Those of layer 5 were equally distributed between inhibited, excited, unaffected, and showing mixed effects. In contrast, most of the layer 6 cells were excited.

The inhibition of layer 4 cells may be presynaptic or postsynaptic, and without intracellular recording of postsynaptic potentials, it is difficult to prove which. Since there is a PT-evoked DRP whose negative phase usually corresponds to inhibition of the cells, it seems likely that this inhibition may be partly presynaptic. Recording intracellularly from neurons excited by cutaneous afferents, Lundberg, Norrsell and Voorhoeve (1962) found no evidence of inhibitory postsynaptic potentials evoked by cortical stimulation.

If the inhibition is presynaptic, the fact that spontaneous activity is reduced as well as responses to peripheral stimulation means that the spontaneous activity is maintained by activity in afferent fibers, not by descending impulses from higher centers, as suggested by Hagbarth and Fex (1959).

Also, if the inhibition seen was presynaptic the fact that some layer 4 cells were unaffected indicates that certain afferent fibers were preferentially depolarized. It is still possible that these cells may have been unaffected only for the range of intensities used; stimulating the PT at more than three time flexion threshold might have re-

vealed inhibition of input to more layer 4 cells. In any case, the results still demonstrate that certain afferent fibers are more strongly affected than others.

It would be interesting to see what other properties distinguish the unaffected layer 4 cells from the inhibited (using the term "inhibited" to include presynaptic inhibition of afferents terminating on the cell). Our results indicate that those showing inhibition are more likely to have a wide dynamic range than those unaffected. They are also more likely to have axons projecting up the dorsolateral column. Another distinguishing property of PT-inhibited cells might be afferent inhibition, but this has not been systematically examined here. This seems plausible since Taub (1963) described weak inhibitory cutaneous receptive fields for most units of the DLC. Also, Gordon and Jukes (1964) found a similar strong correlation between PT and peripheral inhibition in cells of the gracile nucleus, the homologue of layer 4 in terms of afferent connections.

The weak firing seen in some cells at 15-20 ms., preceding a stronger inhibition (Figs. 14+21), may be the postsynaptic correlate of a dorsal root "reflex" -- i.e., if the PT-evoked depolarization of the afferent fibers is sufficiently sudden, it may initiate action potentials, whose orthodromic conduction could excite the layer 4 cells. One might expect the antidromically conducted impulses to be seen on the dorsal root; Fig. 14 shows a few spikes at the beginning of the DRP, simultaneous with the firing of the

layer 4 cell, but this activity is rather weak. If the cell firing is associated with a sudden onset of depolarization, it should be abolished when the depolarization is increased more gradually, by a PT train whose intensity is slowly increased; this has not been tried yet.

To some extent the layer 5 cells seemed to show a mixture of the PT effects seen in layers 4 and 6. The time course and threshold of inhibition resembled that seen in layer 4. Wall (1966) postulated that the layer 5 cells receive their input from layer 4. According to this scheme, the layer 5 cells showing PT inhibition or no effects might be found ventral to layer 4 cells with the same effects. Although such correlations were sometimes found, the number of cases observed is still too small to be statistically meaningful.

The layer 5 cells showing mixed excitation and inhibition by the PT may have received input from similar cells of layer 4, but the period of excitation was often longer in layer 5. The relatively high PT threshold at which both excitatory and inhibitory phases appeared indicates that opposing influences may have been superimposed. The simplest explanation of the behavior of this group is that they received inputs from layer 4 cells or afferent fibers which were inhibited by the PT and simultaneously received excitation from the PT. Alternative interpretations are of course also possible.

The cells of layer 5 which were excited by the PT exhibited no inhibitory phase (Fig. 16); their time course and threshold for excitation was similar to that seen in layer 6. The unit illustrated in Fig. 16 even showed a facilitated response to electrical stimulation of the skin at a time after the PT train when inhibition of other cells was maximum. Their low threshold for excitation and lack of inhibitory effects indicates that these cells may receive their peripheral input via afferent fibers or dorsal cells which escape inhibition.

About three-fourths of the layer 6 cells were excited by the PT. The low threshold and early response of some of these units indicates a potent and possibly direct excitatory action from the PT. Wall (1966) postulated that layer 6 cells receive their cutaneous input from layer 5 cells and their proprioceptive input directly from afferent fibers. The relative scarcity of PT inhibition of cutaneous input in this region may mean that these cells receive cutaneous input via the unaffected dorsal cells. Alternatively, the excitation from the PT may over-ride any inhibition of input.

The PT effects on dorsal horn interneurons may be summarized in terms of two groups of cells. A dorsal group, consisting of layer 4 and some layer 5 cells, contains interneurons which are inhibited or unaffected by PT stimulation. These cells are in close contact with cutaneous afferent fibers and some contribute axons to the ascending DLC. Anatomically, the PT fibers terminating among these cells

are found to arise from the postcruciate "sensory" cortex. In contrast, a ventral group of cells, consisting of layer 6 and some layer 5 cells, is predominantly excited by the PT. Their main cutaneous input is relayed indirectly, via the dorsal group; many also receive direct inputs from muscles and joints. The PT fibers ending among these cells are found to arise predominantly from the precruciate "motor" cortex. Considerable overlap of these groups is found in layer 5.

V. PT effects on DLC cells.

It was interesting to find that the layer 4 and 5 cells sending axons up the DLC were more likely to be inhibited by the PT than those which do not. Of the 19 layer 4 cells which responded antidromically to DLC stimulation, 14 (74%) showed PT inhibition or mixed effects; of the 25 which could not be antidromically fired, only 11 (46%) showed such effects. The difference becomes even greater when layer 5 cells are included. This indicates a preferential inhibition of cells sending impulses up the DLC as compared to those presumably involved in local circuits.

As discussed below, the DLC is an important pathway relaying cutaneous information to higher centers. The response of DLC cells to electrical stimulation of cutaneous nerves has been recently investigated and has led to some interesting theories. Mendell and Wall (1965), recording in

the DLC and stimulating the sural nerve, found that an afferent volley restricted to the large A fibers evoked a repetitive discharge in the DLC units beginning 3-5 ms. after the stimulus and lasting from 8 to 40 ms., followed by a silent period. The silent period was apparently correlated with the reflex depolarization of the afferent fibers and the consequent presynaptic inhibition initiated by the volley. Repeating the stimulus every second reproduced the response sequence. When the stimulus intensity was increased to excite also the small C fibers, the afferent volley evoked additional bursts of activity from 100 to as much as 600 ms. Repeating the stimulus every second resulted in successively longer responses to the C fiber volleys. This late discharge and its increase in length with repeated stimuli could also be evoked by volleys restricted to just the C fibers.

Since previous experiments had shown that volleys restricted to A and to C fibers produced negative and positive DRP's respectively, it became clear that these two fiber groups have opposite long-lasting effects. A volley in the A fibers evoked a reflex depolarization of the afferent fibers and reduced the effectiveness of subsequent volleys. In contrast, a volley in the C fibers inhibited the depolarizing mechanism, thereby enhancing the effectiveness of subsequent volleys. Thus, the balance of activity in A and C fibers was postulated to set the level of a presynaptic

"gate" which determined the transmission of subsequent input.

Our results demonstrate that an additional, descending influence on the level of this presynaptic gate may be mediated via the PT, and acts to decrease the transmission of input. Thus, it parallels the long-range effect of the A fibers in reducing input, but does not have their direct effect of exciting the postsynaptic cells. Fig. 20 shows that the response to both A and C fiber volleys could be inhibited by the PT. Also, a short PT train could produce a brief inhibition of part of the C fiber response, leaving later responses intact. (Fig. 21).

Before speculating on the possible function of such a descending control of the presynaptic gate, we must examine the connections of the pathways involved.

VI. A relation between ascending and descending dorsolateral pathways.

Of the major ascending cutaneous pathways in the cat's spinal cord, the DLC seems at least as important functionally as the dorsal columns. A comparison of the properties of these two pathways at comparable levels (Wall, 1961, Taub and Bishop, 1965) indicates that the DLC transmits a greater variety of modalities, largely due to the wider spectrum of its afferent fibers. The DLC also provides a greater contribution to the evoked response in the reticular formation (Morillo and Baylor, 1963), and is essential for the earliest

phase of the evoked response in sensory cortex (Mark and Steiner, 1958, Norrsell and Voorhoeve, 1962). Norrsell and Voorhoeve (1962) demonstrated that transmissions to the cortex via the DLC was 2 ms. faster than the dorsal columns for electrical stimulation of afferent nerves, and 4 ms. faster for natural hair stimulation. Thus, the DLC seems to form a major pathway for relaying cutaneous information to the sensory cortex.

The fact that the cells of the DLC receive descending inhibition from the cortex suggests the possibility of a negative feedback loop. There is convincing evidence that synaptic linkages are sufficiently effective to form such a loop. As early as 1939 Adrian and Moruzzi observed that natural stimulation could evoke reflex activity in the PT of lightly anesthetized cats. A synchronous afferent volley from a shock to peripheral nerve or skin is even more effective in evoking a reflex PT discharge (Patton and Amassian, 1960, Towe and Zimmerman, 1962). Such a corticofugal discharge recorded in the bulbar pyramids usually begins 10-12 ms. after a shock to the contralateral forelimb, and continues for 15 ms. or more. There is reason to believe that at least the earliest part of this cortical reflex is mediated via the postcruciate "sensory" cortex. Towe, Patton and Kennedy (1964) found that postcruciate PT cells responded on the average 5 ms. earlier to a forepaw shock than those of the precruciate cortex.

Recent evidence indicates that the PT fibers producing presynaptic inhibition arise from the sensory cortex. Andersen, Eccles and Sears (1964) evoked spinal cord DRP's by electrical stimulation of both pre- and postcruciate cortex; however, after ablating these regions they found that DRP's could be evoked only by stimulating the white matter under the sensory cortex. They concluded that motor cortex stimulation evoked DRP's via collaterals to the sensory cortex. In a similar experiment Morrison and Pompeiano (1965) ablated motor cortex and stimulated the PT after axonal degeneration; they could evoke normal DRP's, but no ventral root reflex.

Thus, the evidence supports the possibility of a rapidly conducting negative feed-back loop via the sensory cortex. A sudden cutaneous stimulus would initiate activity which could be transmitted most rapidly via the DLC pathway(s) to the sensory cortex, and via the PT back to the dorsal horn cells inhibiting input to the DLC. The total conduction time would be about 20-30 ms.; thus descending activity could return to the dorsal horn before the arrival of the afferent volley in the fine fibers.

That such a loop is functional under normal circumstances is rank speculation. It would obviously act in conjunction with many other ascending and descending pathways. To describe it as a "loop" may be a misleading oversimplification, considering the many modulating and contributing influences

along the way. The variability of its relays would make such a cortical "reflex" highly labile, and capable of reflecting the states of higher centers.

VII. The dual function of the PT.

Combining our results with those recently obtained by others, it becomes clear that the PT can be considered in terms of two components performing substantially different functions. One component arises from the postcruciate "sensory" cortex and terminates predominantly in the dorsal laminae of the dorsal horn (layers 3-5, Nyberg-Hansen and Brodal, 1963). When stimulated alone, it evokes a DRP (Andersen, Eccles and Sears, 1964) but not a ventral root discharge (Morrison and Pompeiano, 1965). As found here, it seems to inhibit the response of many layer 4 and 5 cells to peripheral stimulation -- probably presynaptically, but possibly also postsynaptically. This inhibition selectively affects certain cells, particularly those giving rise to the DLC, a major cutaneous pathway to the sensory cortex. The cortical cells giving rise to this component of the PT receive a relatively restricted convergence of input, as judged by the preponderance of fixed, relatively small receptive fields in unanesthetized cats (Brooks, Rudomin and Slayman, 1961), and by the restriction of their responses to somatic modalities (Buser and Imbert, 1961). The potent

and rapid transmission of cutaneous impulses to these cells via the DLC pathway suggests the possibility that this component may be involved in a negative feedback loop. In view of its origin and probable function it might be appropriate to call this the "sensory component" of the PT.

In contrast to the sensory component is the "motor component" which arises from the precruciate "motor" cortex and terminates predominantly in the base of the dorsal horn -- laminae 5 and 6 and the most dorsal part of lamina 7 (Nyberg-Hansen and Brodal, 1963). If stimulated alone, this component is capable of evoking a flexion response (Marchiafava and Pompeiano, 1964), but not a DRP (Andersen, Eccles and Sears, 1964). It is probably responsible for much of the excitation of layer 5 and 6 cells found in this study. Most of these interneurons respond to movement of joints and muscles as well as tactile stimuli, and are strongly excited by "flexor reflex afferents" (Eccles, Kostyuk and Schmidt, 1961). The cortical cells giving rise to this component receive a relatively wide convergence of input, as evidenced by their tendency to have wide, labile receptive fields in unanesthetized cats (Brooks, Rudomin and Slayman, 1961), and by their response to diverse sensory modalities (Buser and Imbert, 1961). In view of its origin and termination, and its predominant function, it is probably appropriate to call this the "motor component" of the PT.

It is clearly of interest to re-examine the responses of dorsal horn interneurons to separate stimulation of these

two components. One particular issue which might be settled is the identification of the cells responsible for presynaptic inhibition. Eccles, Kostyuk and Schmidt (1961) implicate the interneurons at the base of the dorsal horn, since they respond to FRA volleys during the rising phase of the DRP. Wall (1962, 1964) favors the cells of layers 2 and 3, on the basis of their position and connections, but cannot prove this by direct recording since they are too small. Although FRA's evoke a DRP, they also evoke a flexion reflex; it is quite possible that Eccles' "D cells" are involved in the latter but not the former. To identify the cells responsible for the DRP, the property that they fire during its generation is necessary but obviously not sufficient. The question may be resolved by stimulating the sensory component of the PT, which evokes only a DRP and no flexion; if layer 5 and 6 cells are not excited by this component, they could not be essential for generating a DRP.

A further investigation of the properties and possible interaction of these two component of the PT is clearly of interest.

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