EXPERIMENTAL NEUROLOGY 55, 469–482 (1977)

Responses of Single Units in Cervical Spinal Cord of Alert Monkeys

MARK B. BROMBERG AND EBERHARD E. FETZ [1]

Departments of Neurological Surgery and Physiology and Biophysics, and Regional Primate Research Center, University of Washington School of Medicine, Seattle, Washington 98195

Received November 17, 1976

Activity of single units was recorded extracellularly in the cervical spinal cord of five unanesthetized monkeys (*Macaca mulatta*), three of which had been trained to flex and extend the right wrist. Units were recorded with tungsten microelectrodes advanced through a chamber mounted over two cervial segments between C_6 and T_1. Unit responses to electrical stimulation of the medullary pyramidal tract and three peripheral nerves of the arm at the brachial plexus were tested with chronically indwelling electrodes. The adequate natural stimuli for each unit also were characterized. Many spontaneously active units were not influenced by natural or electrical stimulation, or during active wrist movements. Units activated by cutaneous stimulation were isolated in the dorsal portion of the cord and most were not spontaneously active. Isolated units with well demarcated cutaneous receptive fields responded to brushing hairs, touching skin, gentle pinching, or some combination of these. Units responding to passive joint movements of the fingers, wrist, elbow, and/or shoulder were isolated at all depths of the cord and most exhibited some spontaneous activity. Many units responded during the phasic components of active wrist movements, and some were driven by comparable passive wrist movements.

INTRODUCTION

The response properties of spinal cord cells have been documented almost entirely in anesthetized, spinal, or decerebrate preparations. The anesthetic agents and ablation procedures typically utilized may significantly affect the spontaneous and evoked activity of spinal cord cells. For

[1] This study was supported by National Institutes of Health Grants NS 04053, NS 05082, NS 11027, and RR 00166. Dr. Bromberg's present address is the Department of Neurology, Columbia University, College of Physicians and Surgeons, 630 West 168 Street, New York, New York 10032.

ISSN 0014-4886

example, barbiturate anesthetics severely depress spontaneous activity and responses to natural and electrical stimulation of cells in both dorsal horn (6, 12) and ventral horn (4). Lamina V cells (10) appear particularly susceptible to anesthetic depression (6, 12). To circumvent the use of anesthetic agents, decerebrate or spinal preparations are commonly used; however, the response properties of many cord cells strongly depend on which descending supraspinal systems remain intact. When decerebrate preparations are made spinal, many dorsal horn cells become less responsive to proprioceptive stimuli and are dominated by cutaneous stimuli (11, 12).

Recording from unanesthetized, intact animals would avoid these problems and permit units to be studied during behavioral responses. Several reports have described the methods and preliminary results obtained from unit recording in the spinal cord of unanesthetized, intact rats (13), cats (7, 8), and tranquilized monkeys (1). The present study was undertaken to document more completely the response properties of cells in the cervical cord of monkeys uninfluenced by anesthetic, tranquilizing, or paralyzing drugs. Isolated units were characterized by their responses to natural stimulation of the periphery, and to electrical stimulation of peripheral nerves in the arm and of the pyramidal tract. In three monkeys trained to make active wrist movements, the response of cells could be documented during active as well as passive movements.

METHODS

Unit activity was recorded in five adolescent rhesus monkeys (*Macaca mulatta*) weighing 4.0 to 5.5 kg. Three were trained prior to surgery to flex and extend the right wrist on a self-paced schedule. With the elbow joint fixed at 90° and the hand held between two padded plates, the monkeys alternately flexed and extended the wrist between mechanical stops for an applesauce reward. All animals were trained to allow passive manipulation of the arm without struggling.

Under sterile conditions we implanted (i) a stainless-steel spinal recording chamber mounted over two adjacent vertebrae between C_6 and T_1; (ii) head stabilization screws over the occipital skull; (iii) fine wire stimulating electrodes in the right ulnar, radial, and median nerves as they emerge from the brachial plexus; and (iv) a bipolar stimulating electrode in the left pyramidal tract (Fig. 1). The implantation and recording techniques described previously (1) were followed with some modification. To assure recording stability, three vertebrae above and two vertebrae below the recording chamber were fused using bilateral pairs of Vitalium screws (4 to 5 mm long) in the vertebral arch to anchor dental acrylic (Fig. 1B). Because the commercially available chamber (Trent Wells) was too short to span the distance from the cord to the dorsal surface of the animal's

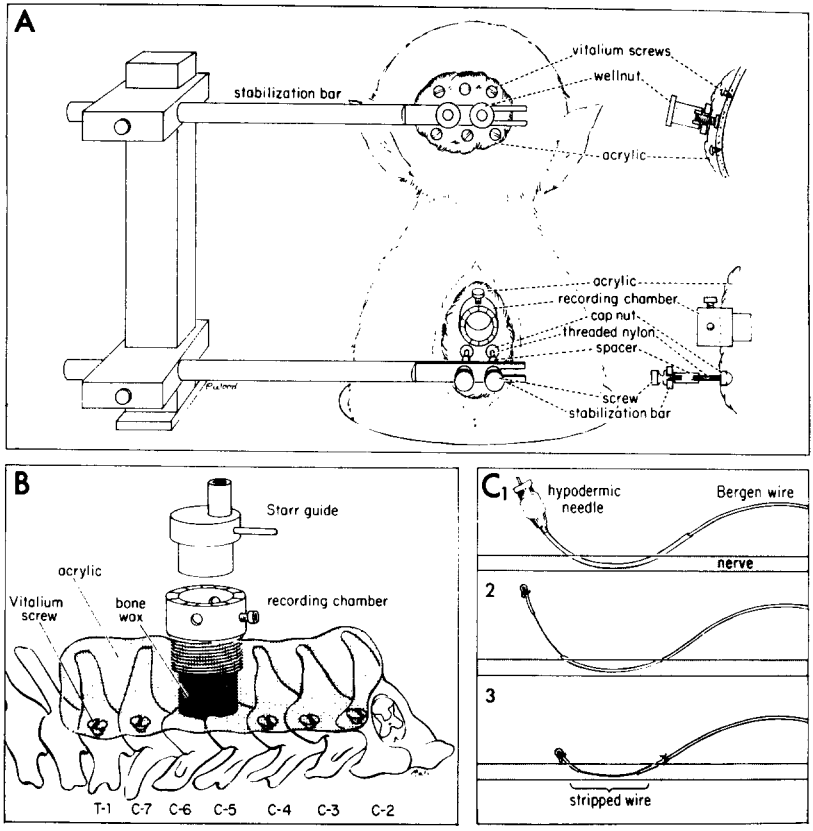

FIG. 1. Diagrams of cervical recording chamber implant and method of peripheral nerve electrode implantation. A—Animal seated in primate chair in recording position, with head and recording chamber held by stabilization bars. Detail of attachment shown at right. B—Details of the recording chamber implant over cervical vertebrae. C—Stimulating electrodes threaded into nerve with the aid of a 23-gauge hypodermic needle. After removing the needle, the uninsulated segment of wire is retracted into nerve.

back, its length was extended by attaching a cylinder of bone wax. When the extended plug and vertebral holding screws were encased in dental acrylic, the wax cylinder formed a protective seal through which the tungsten microelectrodes passed easily.

During recording sessions, the monkey's head was immobilized with a stabilization bar fixed to a post on the chair (Fig. 1A). A second stabilization bar was connected to the spinal recording chamber with flexible nylon threaded rods screwed into cap nuts embedded in the acrylic. This flexible coupling absorbed some of the stresses produced by movement and seemed to prolong the life of the implant.

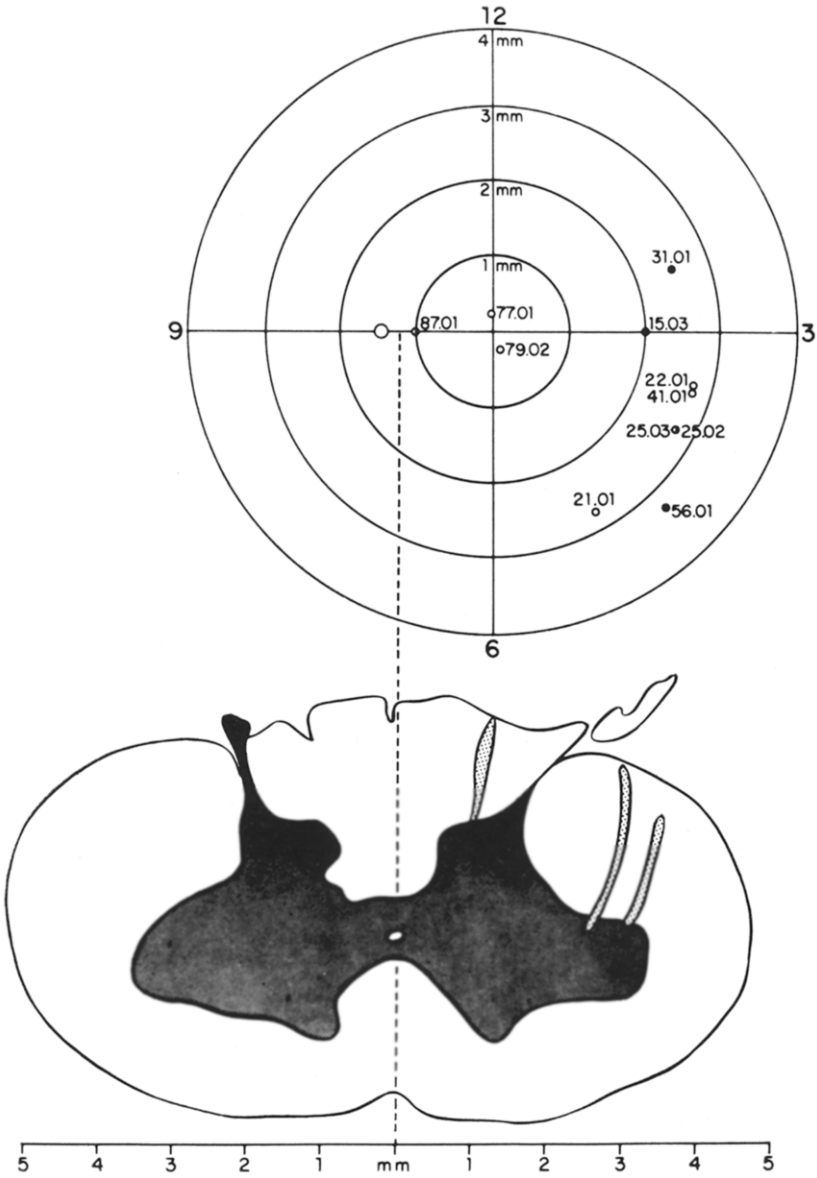

FIG. 2. Polar coordinate map of electrode track sites and cord cross section from one animal. Polar map projects onto dorsal surface of cord. Polar coordinates are the radial and angular position of eccentric Starr guides (cf. Fig. 1). Electrode tracks are marked by large and small circles indicating tracks with cells responsive to stimulation on left and right sides of animal, respectively. Dashed line projects center of cord cross section onto polar map. Open and filled circles mark tracks with cells responding to cutaneous and proprioceptive stimulation, respectively. Numbers refer to some cells illustrated in subsequent figures. Representative traces of electrode tracks are indicated in cord cross section.

Three pairs of insulated wires for stimulating the right ulnar, median, and radial nerves were encased in a single Silastic tube and led from the brachial plexus subcutaneously to a terminal socket secured to the skull. Pairs of insulated, stranded, stainless-steel wires (Bergen Wire Rope Co., Lodi, New Jersey, No. 09.6) were threaded directly into each nerve with a hy-

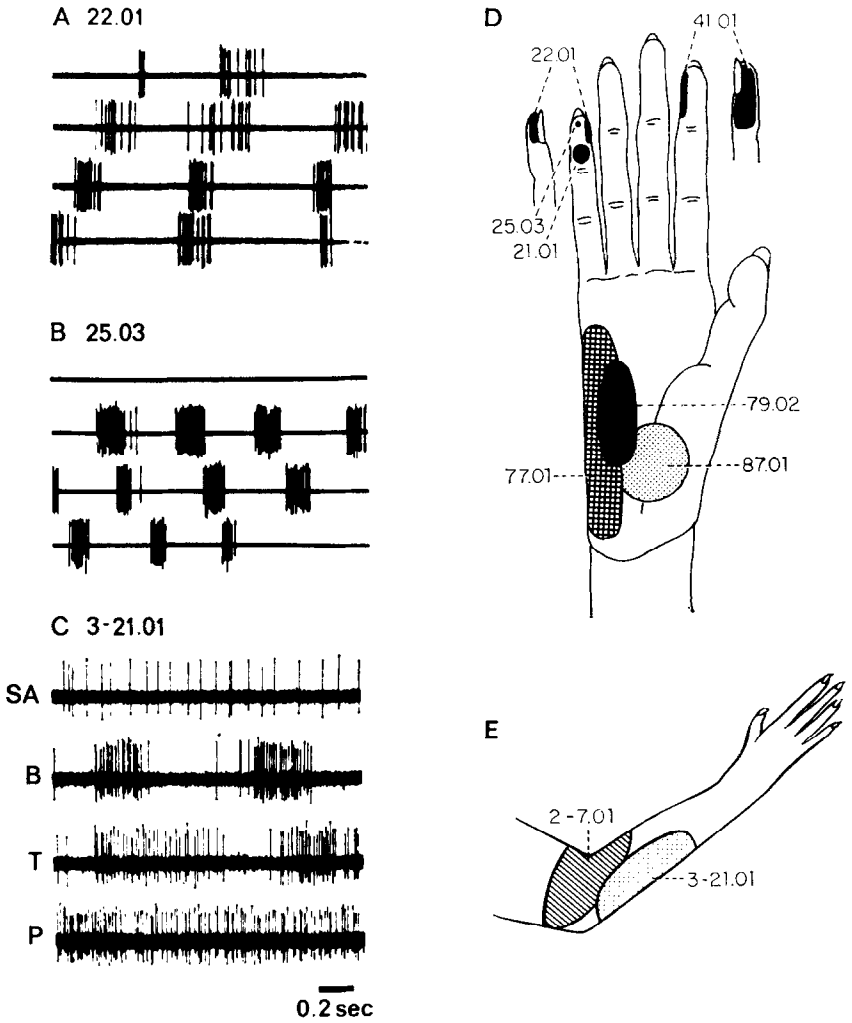

FIG. 3. Responses of cervical units activated by cutaneous stimulation. A—Responses of unit 22.01 to touching the skin. B—Responses of unit 25.03 to touch over small punctate field. C—Unit 3-21.01 exhibited some spontaneous activity (SA); it was activated by touch (T), brushing (B), and adapted slowly to pinching (P). Successive traces in A and B are continuous. D and E—Size and location of receptive fields of cutaneous units.

TABLE 1

Distribution by Forelimb Joints of Adequate Proprioceptive Movement for 47 Cervical Cord Units

	Phalangeal joint	Wrist	Elbow	Shoulder
Flexion	1	6	9	
Extension	2	14	3	
Flexion and extension		2		
Rotation		3		
Abduction–adduction; flexion and extension				7

podermic needle (1) (Fig. 1C). To prevent dislodgement, 4-0 silk suture was tied around the proximal and distal end of each wire. Electromyographic (EMG) activity of forearm muscles was recorded with pairs of wire electrodes implanted either acutely or chronically into wrist flexor and extensor muscles.

Activity of spinal cord units was recorded with 0.2032-mm (0.008-inch) diameter tungsten microelectrodes. The electrodes were advanced by a remotely controlled hydraulic microdrive (Trent Wells) and passed through the intact dura perpendicular to the dorsal surface of the cord. Extracellular action potentials had stable amplitudes and rarely fluctuated during moderate limb movement or in synchrony with cardiac or respiratory cycles.

RESULTS

The sites of electrode penetrations in each animal were mapped with respect to the dorsal surface of the cord by polar coordinates (Fig. 2). Units were detected by their spontaneous activity or by responses to natural cutaneous or proprioceptive stimulation of the right arm. The polar map and sample cord cross section in Fig. 2 are from the same animal and indicate recording sites and modalities of some units discussed below. The midline of the cord was established by determining where cutaneous receptive fields changed from right to left sides. The location of units in the cord was deduced from their coordinates, depth of isolation relative to first signs of unit activity, and response characteristics.

From a total of approximately 250 electrode penetrations, a surprisingly large proportion yielded no units, possibly due to poor electrodes or penetrations into white matter. An average of 1.5 units per productive electrode track were well isolated from background activity. One hundred and twenty-eight units were recorded long enough for characterization; 25 responded clearly to defined cutaneous stimuli, 47 to definable proprioceptive stimuli, and 56 were unresponsive to any stimuli. In addition, another

group of units was driven unreliably by stimuli which could not be characterized clearly as either cutaneous or proprioceptive. The unresponsive, spontaneously active units were intermingled with units activated by specific stimuli. Many unresponsive units did not respond during extensive

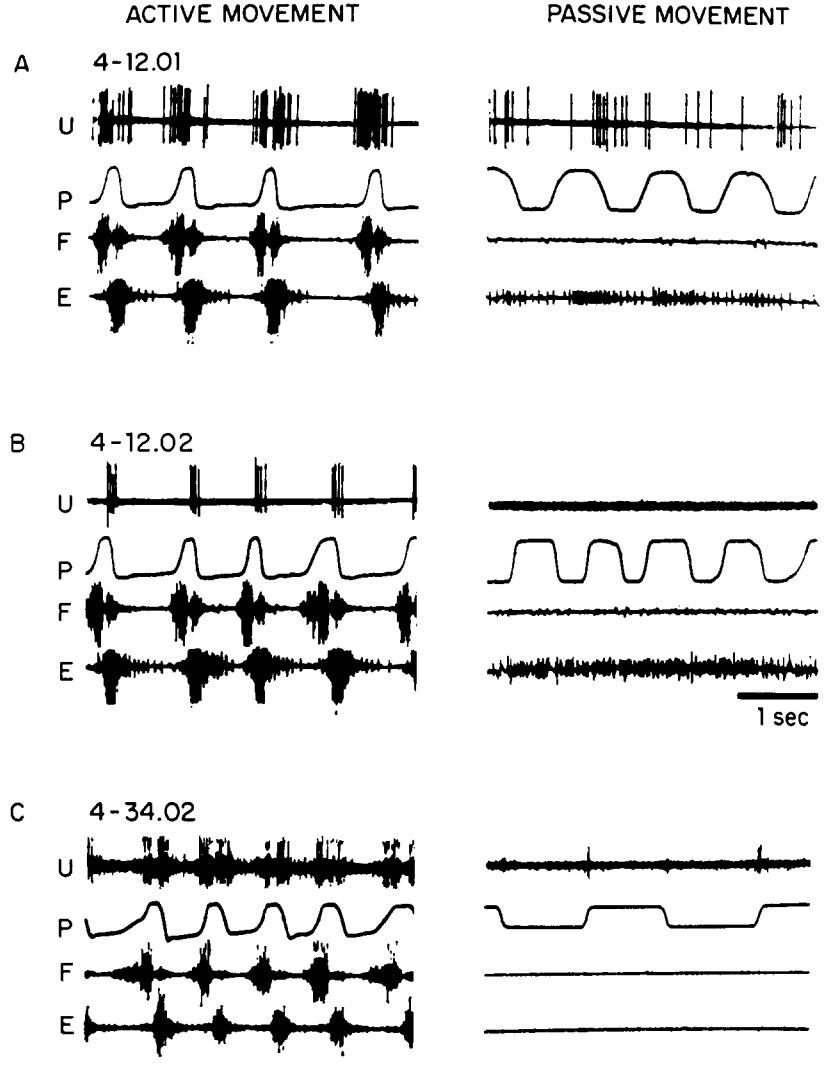

FIG. 4. Response patterns of cervical cord cells during active and passive flexion and extension of wrist. A—Unit 4-21.01 responded during phasic active flexion and extension. B—Unit 4-12.02 responded only during active extension. C—Unit 4-34.02 responded during phasic active flexion and extension, and responded briefly to passive flexion. U, unit response; P, wrist position (flexion upward); F, wrist flexor EMG; E, wrist extensor EMG.

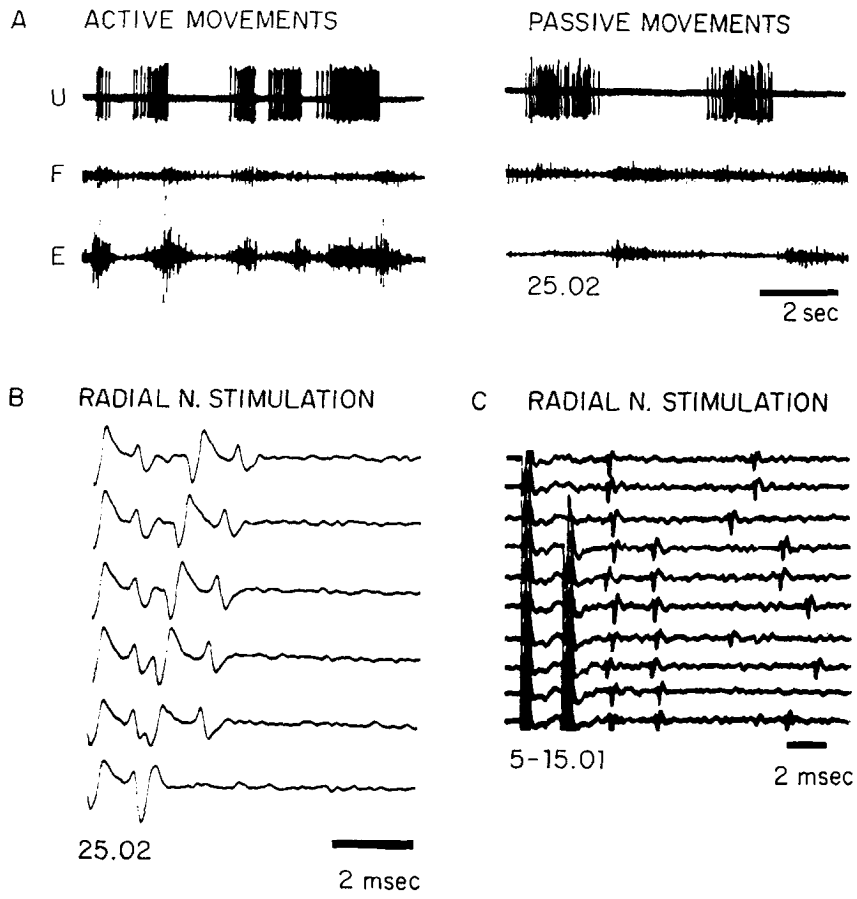

FIG. 5. Unit responses to active and passive wrist movements and responses to radial nerve stimulation. A—Unit 25.02 activity was correlated with active and passive extension; although position was not recorded, the effective directions of movement were repeatedly identified by voice from taped data. B and C—Response of two units to pairs of electrical stimuli delivered to the radial nerve. Unit 25.02 (B) followed two stimuli separated by 1.3 ms. Unit 5-15.01 (C) followed pairs of stimuli separated by 2.1 ms with variable latency.

testing of cutaneous and proprioceptive input nor during general, active movement by the animals. This report describes units activated by specific stimuli and/or active wrist movements.

Units responding to cutaneous stimulation of the right arm were isolated in the dorsal third of the cord (first third of penetration). The natural cutaneous stimuli adequate to drive units were brushing the hairs, touching the skin, or pressure. Cutaneous receptive fields were discrete and had

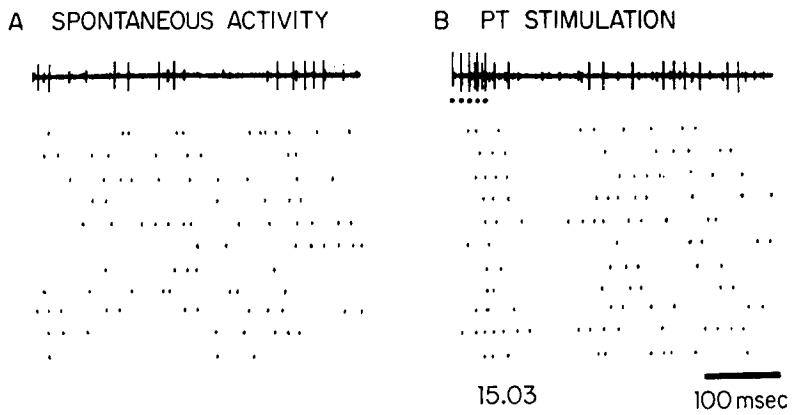

FIG. 6. Effect of a brief train of pyramidal tract stimuli on spontaneous activity of unit 15.03. A—Sample record and continuous dot raster display of spontaneous unit activity. B—Sample and dot raster display of unit activity after five pyramidal tract shocks at 100 Hz; stimulus artifacts marked by dots under top record, but eliminated from dot raster.

well defined boundaries. Proximal receptive fields tended to be larger than distal fields (Fig. 3). Cell 22.01 responded to gentle touching of the skin on the medial side of the fifth digit (Figs. 3A, D), and cell 25.03 responded to punctate touch applied to a very small area of skin (Figs. 3B, D). Some cells were activated by a combination of these stimuli; for example, cell 3-21.01 responded to touching or brushing the hairs over the proximal forearm, and adapted slowly to a gentle pinch (Figs. 3C, E). In the absence of identifiable stimulation, most cells with cutaneous receptive fields were not spontaneously active (the cells in Figs. 3A and B exhibited no activity between responses).

Some units isolated in the initial third of the electrode tracks responded to stimuli applied to the right leg and were probably ascending fibers of the fasciculus gracilis or dorsolateral funiculus. These units were spontaneously active, and were driven by passive and active movements of hindlimb joints; none had cutaneous receptive fields on the leg.

Forty-seven isolated units responded clearly to passive proprioceptive stimulation or to active movement of the right arm; these units were recorded at all depths in the cord. Passive proprioceptive stimulation included manual manipulation about finger, wrist, elbow, and shoulder joints. Active movements by the animals either were unrestrained or were confined to wrist flexion and extension in the manipulandum. More than half of the units were associated with wrist movements, 25% with the elbow, and the remainder with movements of the fingers or shoulder. Four cervical units were activated during some phase of the respiratory cycle; these

responses did not appear to be mediated by periodic electrode injury, because action potentials remained stable. Table 1 lists the distribution of adequate proprioceptive stimulation according to the joints involved. Adequate stimuli were discrete and most specific for distal finger joints and least specific for the shoulder.

Activity of seven cervical units was documented during comparable active and passive movements. Figure 4 illustrates records of unit response, hand position, and EMG activity of flexor and extensor muscles for three different cervical units. During active movements, these and other units discharged repeatedly during the phasic component of the wrist movement; in all cases the earliest EMG activity preceded onset of unit discharge. Unit 4-12.01 discharged during the phasic portion of active flexion and extension (Fig. 4A); the same cell exhibited sporadic activity during passive wrist extension. Unit 4-12.02, recorded 250 μm farther in the same track, was related to phasic active extension, but did not discharge during comparable passive movements (Fig. 4B). Unit 4-34.02 fired during phasic portions of both active flexion and active extension, although during passive movements this cell responded briefly but consistently to passive flexion (Fig. 4C). These units, apparently situated in the intermediate gray and ventral horn, had activity more strongly related to active than to passive movements. Activity from unit 25.02, recorded in dorsal horn, was strongly modulated during both active and passive wrist extension (Fig. 5A).

The response of isolated units to electrical stimulation of peripheral nerves also was tested. One to three nerves (radial, ulnar, and medial), as they emerged from the brachial plexus, were stimulated at intensities which evoked just visible muscle contractions and/or threshold EMG responses. Most of the units responsive to natural stimulation were activated orthodromically with latencies between 1.4 and 6 ms by stimulation of at least one of the nerves. The units unresponsive to natural stimulation could not be driven from any of the nerves tested.

Many units encountered in the lower portions of the electrode penetrations were tested for antidromic response to electrical stimulation of one or more peripheral nerves. The criteria for antidromic activation were a short and invariant response latency, ability to follow pairs of stimuli at short interstimulus intervals, and position in ventral horn. No units tested could be considered unequivocally to have been antidromically activated. Figure 5 illustrates two units that met only some of these criteria. Unit 5-15.01 (Fig. 5C) followed a pair of stimuli separated by 2.1 msec (476 Hz), but exhibited a variable response latency and may have been orthodromically driven. Unit 25.02 (Fig. 5B) followed pairs of stimuli at an interval of 1.3 ms (770 Hz) with a constant latency, but was recorded

in the dorsal part of the cord. To confirm that the microelectrode tracks had passed through motoneuron pools of wrist muscle, the radial nerve of one animal was severed 2 weeks prior to sacrifice. Histological examination revealed numerous chromatolytic cells in the ventral horn in the region of the electrode tracks.

Many cervical cord cells also were tested for their response to repetitive electrical stimulation of the pyramidal tract, using trains of four to ten stimuli at 100 and 200 Hz and intensities up to those which evoked just visible muscle contractions or EMG activity. Consistent with previous findings under comparable conditions (1), only four of 17 cells tested were excited, and several cells showed an inhibition of spontaneous activity lasting up to 100 ms. The unit in Fig. 6 responded to a train of five pyramidal tract stimuli at 100 Hz with a moderate burst of activity, followed by suppression of activity for 100 ms. This cell could not be driven by natural stimulation.

DISCUSSION

Although single-unit activity has been recorded extensively in alert, behaving animals in regions of the nervous system rostral to the obex, chronic unit recording in spinal cord has been relatively neglected. The greater technical difficulties of recording spinal units in moving animals appear to be surmountable (1, 7, 8, 13). Sufficient recording stability has been achieved in spite of respiratory and cardiac pulsations and moderate movements by fusing two to three vertebrae above and below the recording chamber. Several vertebrae between the implant and the skull may remain unfused, allowing unrestricted head movements between recording sessions. Fusing the vertebrae around the recording chamber with screws in the lateral processes to anchor dental acrylic provides stable recording for many weeks. Nevertheless, in comparison to the skull, vertebral bone offers a less secure base, is subject to greater and more prolonged stresses, and is more susceptible to osteomyelitis. These factors have limited the practical lifetime of our spinal implants in monkey to 5 or 6 weeks. As previously reported (1), chronically indwelling stimulating electrodes in peripheral nerves where they emerge from the brachial plexus exhibited stable thresholds for evoking EMG responses for many weeks. Stimulus intensities at or below EMG threshold were well tolerated by the awake monkeys.

In the alert monkey, only half of the cervical units adequately tested exhibited clear responses to natural cutaneous or proprioceptive stimulation. Many spontaneously active cells could not be driven reliably by any peripheral stimulation; moreover, many of those appeared to have no relation to active movement made by the monkey. Such unresponsive units were

intermingled with units influenced by peripheral stimulation. Some unresponsive units may have been descending or ascending axons. Their peripheral input, if any, may have come from visceral or high-threshold receptors, or regions not tested. Also, it is conceivable that in the intact, alert animal many spontaneously active spinal cord cells are more strongly controlled by central pathways than by peripheral input.

Units which responded to cutaneous stimuli had receptive fields similar in size and modality to those reported for dorsal horn cells in other studies (2, 9, 11, 13, 14). The majority of units with cutaneous input were not spontaneously active, in agreement with previous observations in unanesthetized animals (1, 13). In anesthetized and decerebrate preparations, cells in lamina VI received both cutaneous and proprioceptive input (11); relatively few units in this study were activated by both cutaneous stimuli and passive joint movement. Most units which were modulated by proprioceptive input responded to passive movement of one or two adjacent joints. The effective movements were most specific at the distal joints (e.g., flexion of a single phalangeal joint), whereas units driven from more proximal joints often responded to several movements (i.e., abduction and extension of the shoulder).

In the monkeys trained to flex and extend the wrist actively, unit discharge could be documented during comparable active and passive movements. Most units related to active wrist movements fired repeatedly during the phasic component of flexion or extension, or during both. None of those cervical cells changed their activity prior to the earliest sign of EMG activity. Unit activity was more intense during active movement than during comparable passive movements. No simple, general relation between effective direction of active and passive movement was found. Some units responded during active and passive movement in the same direction, as would be consistent with input from joint receptors. Others fired during both active flexion and extension, but responded primarily to passive movement in one direction (Figs. 4A, C). A variety of input–output relations was observed for different cells, similar to observations in rostral regions of the motor system (3).

Electrical stimulation through indwelling electrodes in peripheral nerves was intended to measure the convergence of afferent input and to identify motoneurons. Cells with cutaneous receptive fields often responded to electrical stimulation of one or more nerves, including nerves innervating regions outside the receptive field [cf. (12)]. Although many neurons in the ventral portion of the cord were closely related to active wrist movements, no convincing examples of antidromic responses in motoneurons were found in this study. Several reasons may account for this. Because the animals were awake, we preferentially sampled active cells, rather than searching

for those responsive to electrical stimulation as is commonly done in acute preparations. Electrical stimulus intensities did not exceed threshold levels for evoking EMG rsponses, so only the largest motoneurons would have been stimulated. However, relatively few of the large motoneurons would have been active under our recording conditions, because the animals either sat quietly or moved against low inertial loads. Any motoneurons recruited under those conditions would be expected to be small (5) and less likely to be isolated or electrically stimulated. Finally, the criteria used to identify isolated cells as motoneurons were relatively strict. The frequency-following ability of cells in the cord should be interpreted cautiously, as exemplified by the findings shown in Fig. 5C. This unit followed pairs of stimuli at frequencies of 476 Hz, but with variable latency, suggesting the existence of very secure monosynaptic linkage in the cord. Faithful frequency following also may occur in afferent fibers, as may have been the case for the unit in Fig. 5B which was recorded in the dorsal portion of the cord. The most convincing demonstration of an antidromic response is collision with an orthodromic action potential. Another proof of motoneuron recording would be a one-to-one following relationship between unit discharge and peripherally recorded muscle unit activity; the improbable coincidence of simultaneous recording of related neural and muscle units was not encountered in this study.

The limited range of pyramidal tract stimulation intensities also may explain the low proportion of cells influenced by trains of pyramidal tract stimuli, a finding consistent with previous observations under similar conditions (1). In a different animal preparation, a large proportion of dorsal horn cells was modulated by pyramidal tract stimulation when intensities up to three times flexion threshold were used (2).

In conclusion, the experimental techniques described make it possible to record single-unit activity in cervical spinal cord of alert, behaving monkeys for many weeks, and to characterize unit responses to natural and electrical stimulation of peripheral nerves and pyramidal tract, and during trained wrist movements. Limitations in the stimulus intensity range which can be used in the alert animal preclude complete characterization of cell responses to natural stimulation of high-threshold receptors and electrical stimulation of small axons. Nevertheless, chronic recording provides an opportunity to test the function of spinal cord cells under relevant behavioral conditions. The active wrist movement task used in these experiments involved only a portion of the cervical cells recorded. To activate cells unrelated to pretrained wrist movements, an attempt was made to train one monkey to fire cervical units by operantly reinforcing increases in unit activity (3). This monkey soon learned that sufficient struggling against the restraints often produced reinforcement, triggered by a burst of in-

jury discharge. Future attempts to operantly condition spinal cord cells may have to use differential schedules to prevent development of such a strategy.

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