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Unit responses recorded from cervical spinal cord of awake monkey*

KENNETH R. COURTNEY AND EBERHARD E. FETZ

Departments of Neurological Surgery, Physiology and Biophysics, and Regional Primate Research Center, University of Washington School of Medicine, Seattle, Wash. 98195 (U.S.A.)

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The ability to record activity of spinal cord cells in an awake animal for prolonged periods of time is a prerequisite for investigating the function of these cells in a behaving animal. Progress toward this goal has been achieved recently in a preliminary study of responses of cervical spinal cord cells to peripheral and pyramidal tract (PT) stimulation in the monkey. A description of these new recording techniques and some sample data follows.

This technique involved chronic implantation of a stainless steel adaptor over a lower cervical spinal cord segment with fusion of two adjacent vertebrae both rostral and caudal to the adaptor. During the recording sessions the adaptor held a Trent–Wells remotely controlled hydraulic microdrive which advanced tungsten microelectrodes into the spinal cord. With the aid of a broad spectrum antibiotic (*e.g.* chloromycetin), the chronic implants were maintained relatively free of infection in 4 *Macacca mulatta* monkeys (2.5–4.5 kg) for periods of 4–6 weeks. The operated monkeys were housed in a primate cage and chaired daily for the recording sessions. During most recording sessions the animals were awake but sedated (either with phencyclidine HCl [Sernylan], 1 mg/kg, or diazepam, 5 mg/kg). The animal's head was immobilized with a stabilization bar held by a post on the primate chair and fitted into a stainless steel tube implanted over occipital skull (Fig. 1A). The usual neck restraint plates on the chair were removed to leave room for access to the cervical implant. The cervical implant was fixed to the chair by lateral bars as indicated in Fig. 1A in order to achieve sufficient stability for long-term cell recording.

The depth of the commercially available adaptor was extended by a dental acrylic cylinder to a total length of 3–4 cm. During sterile surgery under halothane anesthesia the cord segment of interest (usually C_7, found under the sixth cervical vertebra) was exposed. Stainless steel screws were tapped into the lateral processes of two rostral and two caudal vertebrae. With the recording plug in place dental acrylic was poured over the 8 tapped screws, thus fixing the vertebral implant in place. In closing, the wound cavities were eliminated by carefully suturing muscles and skin in

* Reprints may be obtained from the Primate Information Center SJ-50, University of Washington, Seattle, Wash. 98195, U.S.A.

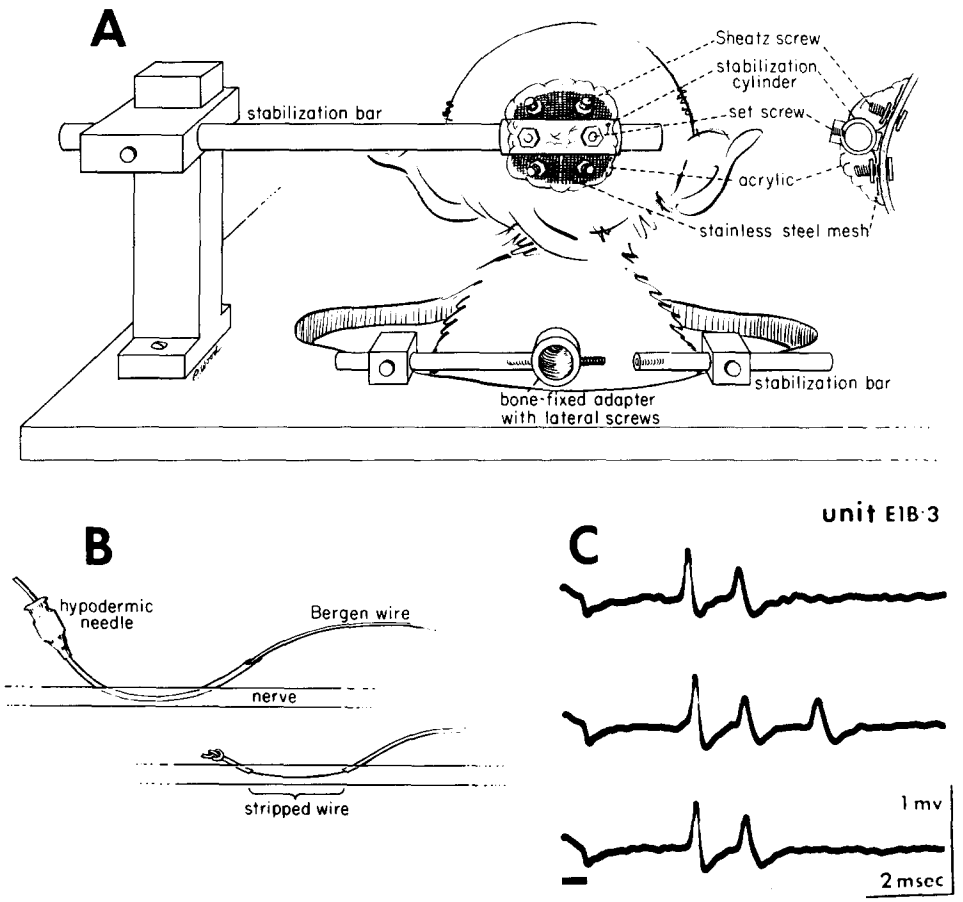

Fig. 1. A, Posterior view of head stabilization arrangement and lateral stabilization of neck implant. B, Technique for implanting stimulating electrodes in nerve, before (above) and after (below) removal of hypodermic needle. C, Response of unit EIB-3 evoked by a pair of median nerve stimulating electrodes at shock strength insufficient to produce observable motor response.

order to preclude potential pockets for infection. The surgical wound was also irrigated with an antibiotic during closure.

In a surgery prior to the spinal implant, we implanted (i) a bipolar pyramidal tract electrode (at stereotaxic coordinates posterior 2, lateral 2), (ii) the skull stabilization tube, and (iii) paired stimulating electrodes in brachial plexus nerves. The terminals of 2 pairs of stimulating electrodes were cemented just below the stabilization implant and from these terminals flexible nylon insulated braided stainless steel wires (12 mil outside diameter, ordered from Bergen Wire Rope Company, Lodi, New Jersey, number BWR 09.6) were led subcutaneously to the brachial plexus, where each pair of wires was threaded into the medial and radial nerves using the following technique. A curved hollow hypodermic needle (21-gauge) was carefully passed under the nerve sheath for a distance of 3–4 mm and the Bergen wire was threaded through the lumen of the needle (Fig. 1B). Then the needle, with the enclosed wire, was pulled back out

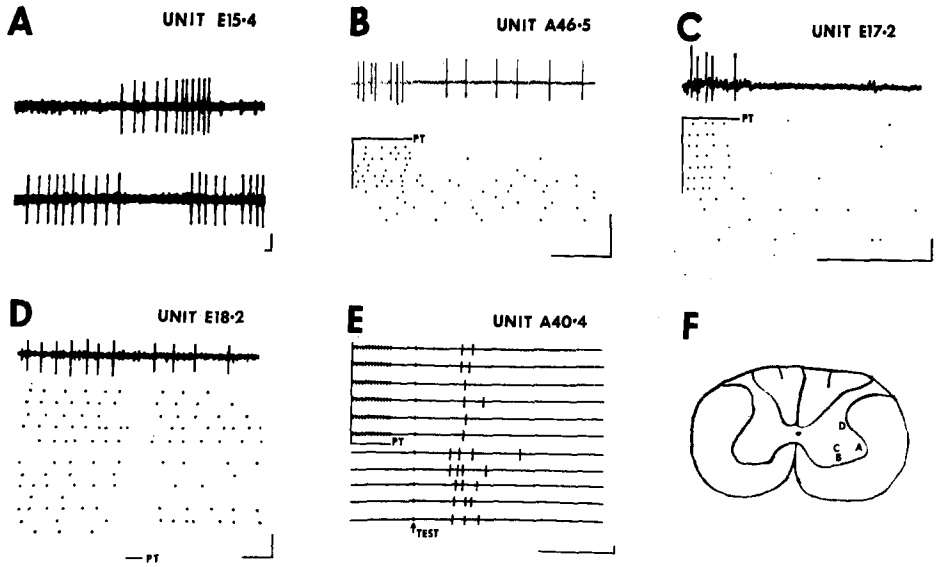

Fig. 2. A, Traces of unitary activity during successive voluntary arm movements demonstrating recording stability. B and C, Responses of units excited by PT stimulation. D, PT inhibition of naturally evoked activity. E, PT inhibition of electrically evoked activity. F, Cell locations as confirmed by electrolytic lesions (unit E was not marked directly but believed to be very near position D). All time scales are 50 msec and voltage scales are 500 μV.

of the nerve leaving a length of wire threaded in the nerve. A knot was tied on the distal end of the wire and 2 mm of insulation removed just proximal to the knot. Finally the wire was gently pulled back into the nerve so that the bare portion was encased inside the nerve (Fig. 1B). Such implants proved less traumatic over time than implants involving external stimulating electrodes kept in contact with the nerve by a Silastic or a rubber cuff. With care taken to avoid surgical nerve trauma and subsequent infection, these stimulating electrodes lasted for many weeks without observable motor impairment of the animal's forelimbs. Stimulus thresholds for evoking visually observed muscle twitches were found to be essentially constant for periods of 4 weeks. Fig. 1C depicts the response of a unit being excited by such median nerve electrodes at a shock strength well below the level necessary to evoke an observable muscle twitch. This unit was located in the dorsal horn and was driven most effectively by touch of the ulnar side of the hand.

Using insulated tungsten microelectrodes, we recorded responses from single cells for tens of minutes, occasionally for more than an hour. Action potentials remained stable even during moderate limb movements (Fig. 2A) and rarely showed signs of fluctuation with respiratory or cardiac cycles. Electrolytic lesions (10–20 μA for 15 sec) were considered to be essential for successful reconstruction of cell location within the cord. We were primarily interested in characterizing responses of spinal cord cells to natural peripheral stimulation (brushing hairs, pressing skin and moving joints) as well as to pyramidal tract stimulation (25–50 msec train at 500/sec).

Fig. 2A shows the activity of a ventral horn unit during voluntary arm move-

ments by the animal; the bursts occurred with 3 successive active movements of the ipsilateral arm. The stability of the action potentials during moderate active limb movements was quite reliable, as long as the monkey did not strain against the stabilization bars. This unit (and unit 2B below) did not respond to radial nerve stimulation at strengths sufficient to evoke muscle responses.

Figs. 2B and 2C depict responses of cells excited by PT stimulation. The responses in Fig. 2B were recorded from a ventral horn cell with a high rate of regular spontaneous activity; the unit responded to pressure on the ipsilateral or contralateral hand. Deep pressure applied to the ipsilateral arm and forearm also excited this unit. The illustration shows a burst of spikes evoked by the 50 msec PT stimulus train and suggests a post-stimulus depression of the ongoing spontaneous activity. Fig. 2C shows the responses of another ventral horn unit to a 25 msec PT train; this unit did not respond to natural stimulation but fired in relation to the animal's active limb movement. This unit responded to weak radial nerve stimulation (*i.e.*, at intensities below threshold for muscle responses) with a variable latency of 2–10 msec.

Fig. 2D illustrates responses of a unit in the base of the dorsal horn which was driven by pinching, but not by touching the skin of the upper arm, particularly the area overlying the triceps muscle (a response to deep pressure of the arm could not be excluded). The figure shows PT inhibition of cell responses evoked by squeezing the upper arm. This unit responded to electrical stimulation of the radial nerve at latencies of 5.5–6 msec. The PT train also diminished the number of spikes evoked by this electrical stimulation (not illustrated).

The unit whose responses are illustrated in Fig. 2E showed no spontaneous activity and responded to skin and hair stimulation of the whole ipsilateral forelimb and adjacent trunk regions, but not to contralateral stimulation. The unit's response

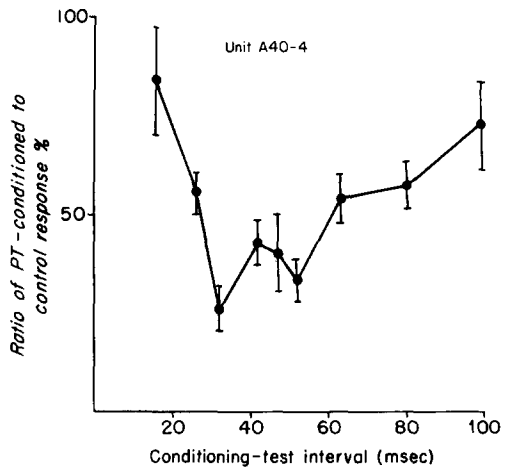

Fig. 3. Ratio of PT conditioned to unconditioned response magnitude as a function of the interva between the start of the conditioning PT train (500/sec, 25 msec duration) and the test shock to skin. Each point represents the ratio of the mean number of spikes with and without PT conditioning in several serial repetitions of the experiment illustrated in Fig. 2E; lines represent ± 1 standard error.

to sustained natural stimulation adapted slowly but completely. PT stimulation (25–50 msec trains) did not noticeably affect naturally evoked activity but did reduce the number of spikes evoked by electrical stimulation of the forearm skin. Fig. 2E indicates the conditioning–testing (C–T) procedure. A conditioning PT train of 25 msec duration preceded the test skin shock by a variable C–T interval (42 msec in Fig. 2E). The decrease in the number of spikes evoked by skin stimulation and the increase in latency of the first spike suggest an inhibitory effect of the PT. At this intensity the PT stimulus train did not cause any perceptible motor response.

Fig. 3 is a graph of the relative number of spikes evoked by the skin shock (relative to control) as a function of the C–T interval. The time course of the inhibitory effect of PT stimulation is similar to the time course of PT-evoked dorsal root potentials and dorsal horn cell inhibition in spinal cats[1]. Besides reducing the number of spikes evoked by the skin shock, the PT conditioning train also increased the latency of the first spike. For example, the latency increased by 21% at the 42 msec C–T interval (Fig. 2E), and by 15% at 80 msec C–T interval ($P \leqslant 0.01$).

As illustrated in Fig. 2F, units influenced by PT stimulation were located in regions receiving terminations of pyramidal tract fibers. Anatomical studies show numerous PT terminals in dorsal as well as ventral horn[2]. Nine of 53 units tested for PT influence in this study were excited by PT stimulation and were clearly located in the ventral horn. Only a few cells exhibited PT inhibition of spontaneous or naturally evoked activity, and half of these were found in more dorsal locations (*cf.* relative distribution of PT effects in ref. 1). However, few cells were tested with respect to PT inhibition of electrically evoked responses, which was found to be a more sensitive test of PT inhibition, as illustrated by cell A40-4 in Figs. 2E and 3. Numerous units unresponsive to PT stimulation were recorded in regions caudal to the cervical enlargement. Unresponsive cells might have been influenced at PT intensities above those used here — *i.e.*, above threshold for evoking movements.

Wall, Freeman, and Major reported success in recording spinal cord units in chronic rats, using a permanently mounted microelectrode drive capable of making a single track[4]. Pompeiano has also reported results using a chronic implant in cat spinal cord[3]. Our observations suggest the feasibility of unit recording in the primate spinal cord with a removable microdrive allowing long-term study of a 5 mm diameter cylinder of the spinal cord. Unit isolation proved surprisingly stable, even during moderate limb movements (Fig. 2A), suggesting that this technique could be used to study unit responses during behavioral tasks.

In summary, we have confirmed the feasibility of chronically implanting in the monkey a microdrive holder over the C_7 vertebral segment, stimulating electrodes in brachial plexus nerves and effective stabilization devices. Using these implants we studied responses of spinal cord cells to peripheral and PT stimulation in sedated but awake monkeys over periods of several weeks.

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