

Long-Term Unit Recording from Somatosensory Neurons in the Spinal Ganglia of the Freely Walking Cat

Abstract. A new technique has been developed for stable, long-term recording from groups of individual primary afferent neurons in the freely walking cat. A number of fine, flexible wires are inserted into dorsal root ganglia via a small laminotomy in the lumbar spine. The cut end of each wire can record stable and separable action potentials from one to three dorsal root ganglion neurons; each unit has typically held for 1 to 4 days. A broad range of myelinated somatosensory afferents (conduction velocities of 30 to 120 meters per second) have been studied during locomotion. Most cutaneous and proprioceptive afferents studied have been sensitive monitors of complex combinations of step-cycle components, and their firing patterns would often have been difficult to predict from existing information.

Recent progress in the study of motor systems has been greatly facilitated by the introduction of long-term single-neuron recording, as, for example, from neurons in the motor cortex of monkeys performing tasks (1). No comparable technique has been developed for monitoring primary somatosensory feedback, known to be vital to the fine control of

locomotion, although short-term records have been obtained for the specialized movements of mastication in the intact animal (2) and fine finger positioning in man (3). Previous attempts to record for a long period from peripheral nerve or dorsal root fibers in walking animals have produced short-term single- or multiple-unit records only from the

largest myelinated proprioceptive fibers (4). Studies of afferent input during "automaton" walking, as in the acute mesencephalic cat preparations (5), are less than satisfactory because of the necessity for restraining the animal and abnormal supraspinal activity. We now present a method for long-term recording of activity from individual dorsal root ganglion (DRG) afferent neurons in a freely walking cat. Myelinated afferents with conduction velocities from 30 to 120 m/sec have been routinely held for 1 to 4 days, with new units usually replacing those which have drifted away. As many as seven microelectrodes have been inserted into one DRG, and each typically records from one to three units with distinctive waveforms, which can be studied separately through the use of a window discriminator (6). The DRG site, although somewhat awkwardly situated in the spinal canal, has the advantage of containing large cell bodies (40 to 80 μ m) packed tightly together in a tough stabilizing matrix of connective tissue. Relatively large but flexible electrode wires (having large tips, low impedance, and excellent noise immunity) can readily record large extracellular unit action potentials from several cell bodies (7).

Figure 1 shows a typical preparation and the surgical technique for fixing and implanting the 50- μ m (diameter) insulated wires (8). The wires are passed through a shielded, flexible Silastic tube, which is fixed by sutures through holes drilled in the spinous process. The wires are cut at an oblique angle to expose a beveled tip, which is pushed by hand through the dural-epineurial sheath into the ganglion while the tip impedance is being monitored. A small amount of slack is left to take up the sliding movement undergone by the DRG during walking because of normal traction on the sciatic nerve. The electrodes "float" in the DRG (9) and sample units at random from a population that appears to

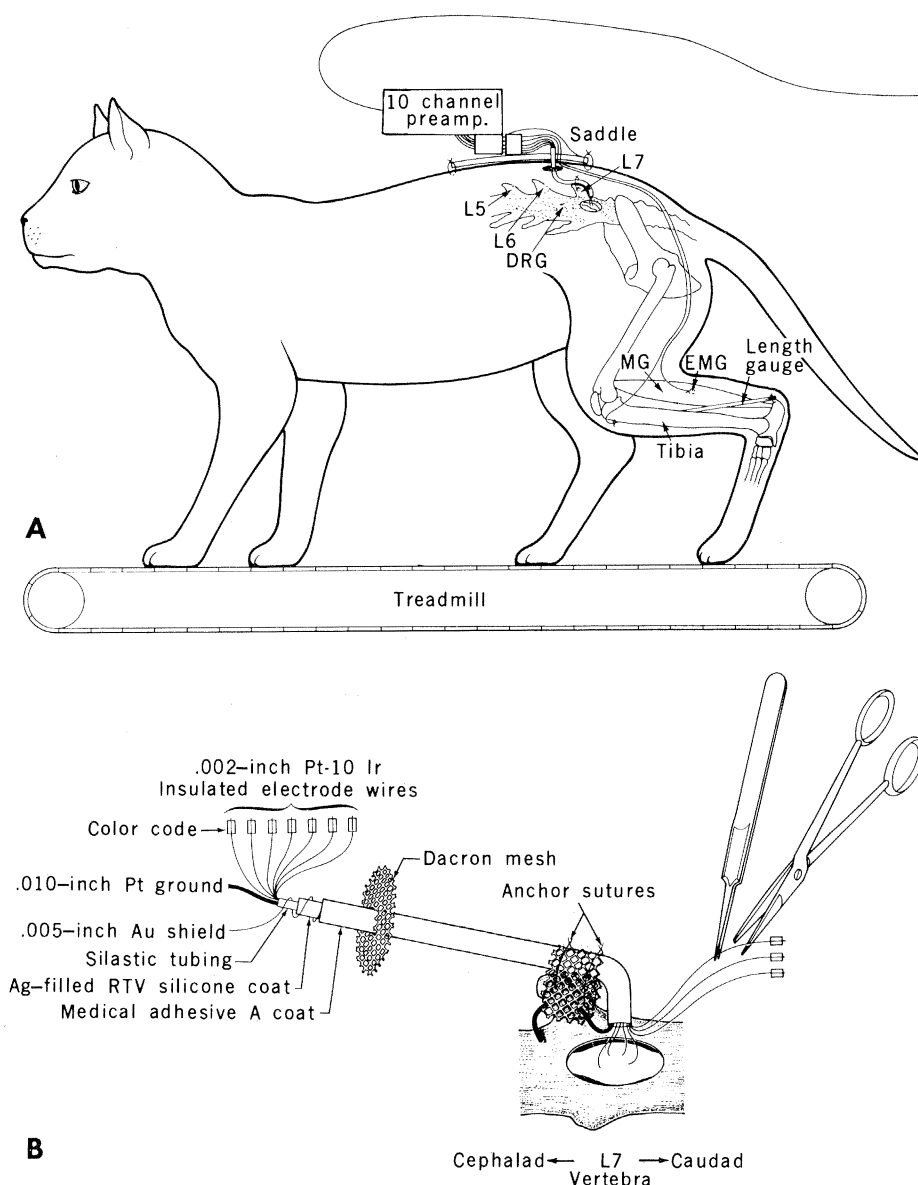


Fig. 1. (A) Long-term unit recording from dorsal root ganglion (DRG) cells. Drawing approximately to scale showing the preamplifier and flexible connector, which is sutured to the lumbar fascia. Leads pass subcutaneously from seven DRG microelectrodes, an implanted ankle-joint angle transducer (19), and an electromyographic electrode sewn into the medial gastrocnemius muscle (MG) to a percutaneous "saddle" connector for pre-amplification. (B) Anchoring of the DRG electrode assembly to the dorsal spine of the seventh lumbar (L7) vertebra and the manual preparation and insertion of the flexible wire microelectrodes into the L7 DRG through the small bur hole. No adhesives or other fixation techniques are used on either the microelectrodes themselves or the DRG.

have little or no spatial or sensory modality organization (10). After the laminotomy incision is closed, the saddle connector is sewn through the skin to the fascia on either side of the incision, and the electrode leads are soldered to the connector and potted in Medical Adhesive A. Such saddle assemblies are well tolerated for weeks without any sign of infection or discomfort to the cat (11). The laminotomy is minimal and the dura is not opened; the animals usually walk normally in 24 to 48 hours without any limp that is visible on videotape recordings.

On each day after the operation, a multichannel frequency-modulated (FM) tape recording is made of the signals from the microelectrodes, electromyographic (EMG) electrodes, and other implanted transducers (Fig. 1) along with a synchronized videotape recording of gait at various treadmill speeds and with various perturbations (for example, upgrade walking and electrical stimulation). The animal is then lightly anesthetized with ketamine, and the units on each electrode are identified by manipulation and electrical and mechanical stimulation of the leg. Small dissections may be undertaken to further identify units, obtain conduction velocities, or implant additional EMG electrodes. Additional electrodes can be connected to unused amplifier channels during the next day's run to provide further information on proprioceptors related to the muscle. Spike amplitude and shape are usually stable and distinctive, permitting reliable reidentification of units from day to day (12).

Figure 2 shows the simultaneous unit activity of three DRG cells during walking. Two of the units were recorded from one electrode (K-X5) and separated by spike shape as shown in the unit records at the left. Unit shape and amplitude were essentially unchanged 12 hours later when the receptors were characterized during a dissection under pentobarbital anesthesia. During locomotion, each unit in Fig. 2 responded consistently during certain phases of each step cycle.

The two guard hair-cell receptors (13) (Fig. 2) exhibit sensitive and stereotyped responses that would be difficult to predict. Unit K-X5A (from L7 DRG) was a reliable indicator of footlift and -fall, despite its location on the dorsum of the toes, probably because of small rubbing motions between hairs on adjacent toes. Unit K-Y7A [from the DRG at the first sacral spinal level (S1) in the same animal] had a receptive field on the plantar surface between the central and lateral

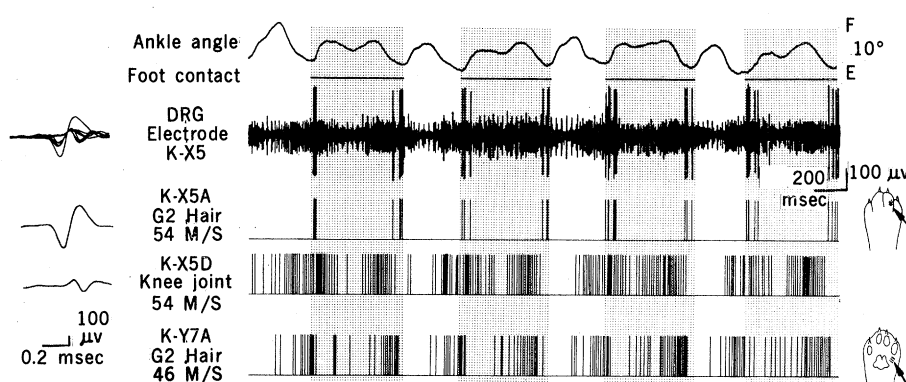


Fig. 2. Activity in three DRG afferents in a freely walking cat, prepared from multichannel FM tape recordings during videotaped treadmill walking. Ankle angle (F , flexion, upward deflection), calibrated from videotape stills, showed small variations among the four step cycles. Foot contact (stance) was taken from the videotape and is indicated by shading across all traces. The L7 DRG electrode K-X5 recorded several discriminable unit potentials as shown at left, with several superimposed action potentials [obtained through the window discriminator (6) a digital trigger and delay line] from each of three units active during the four step cycles shown. The largest unit, a hair-cell receptor, is shown in the detail at the left, and discriminator output in trace K-X5A with the receptive field indicated by the dotted area (arrow) on the line drawing (far right). One of the two smaller discriminable units was the knee-joint receptor K-X5D. The bottom trace is another hair-cell receptor, K-Y7A, discriminated from unit activity recorded simultaneously from another microelectrode in the S1 DRG; note activity during the swing phase when receptive field (dotted area of line drawing, marked by arrow) was not in contact with any object.

toe pads, yet it generated much of its activity during the late swing phase and had variable silent periods while the foot was on the ground (stance phase of gait) (14). Such sensitive and reproducible responses in rapidly conducting fibers might be useful for signaling timing information from step to step but might be labile from day to day as hairs grow, shed, or are damaged.

Joint receptors, while exhibiting little sensitivity to midrange joint angle changes alone, are sensitive to the combination of joint position and capsule tension produced by inserting muscles (15). Unit K-X5D was a knee-joint receptor identified under anesthesia by dissection and stimulation of the posterior articular nerve (16). The firing pattern of this unit during walking could not be explained by a simple consideration of knee-joint angle, joint loading, or velocity of movement alone. Knee extension in the late swing phase was accompanied by an accelerating discharge and followed by a variable burst just after footfall. Small fluctuations in ankle angle (which are necessarily reflected in knee angle during stance) were clearly reflected in the firing pattern during the stance phase of each step cycle. The unit could be modulated from 0 to 200 pulses per second by movements of less than 5° even when we partially unloaded the knee by supporting the hips. Two other knee-joint receptors studied responded to very small external rotations of the joint (independent of flexion-extension and before appreciable resistance was

encountered); one had almost no activity during walking, and the other was smoothly modulated during stance over the range 0 to 150 pulses per second (17).

Our records of muscle receptors are still too scattered among the many hindlimb muscles for us to state general conclusions beyond agreement with Prochazka *et al.* (4), that spindle primaries are apparently responsive to both passive stretch and, more variably, coactivation of the alpha and gamma motor systems.

Although most units showing any activity during walking were modulated by some aspect or aspects of the step cycle, others appear to respond to nonlocomotory rhythms. Proprioceptors from the lower back and hip can respond in phase with respiration, even during locomotion. Rhythmic bursting uninfluenced by any apparent physiological function (for example, pulse, respiration, attentiveness, and gait), by mechanical stimuli (including visceral pressure), or by anesthesia has been seen.

To date, a total of 67 units from 11 cats have been identified (52 from L7, 15 from S1 DRG). Most of the units on which conduction velocity was measured were in the range 30 to 120 m/sec, but we have identified one cutaneous A-delta class fiber (7 m/sec) and one cutaneous C fiber (0.8 m/sec). We suspect that some of the unidentified spontaneously active cells (for example, those showing rhythmic bursting) (ten total to date) may have been sympathetic, thermal, or other small afferent cells with free endings,

and that other inactive sympathetic or high-threshold slow afferents may have been overlooked. Of the physiologically identified units, 33 were cutaneous (18 hair, 8 pressure, 7 other) and 24 were proprioceptive (11 spindle primaries, 4 spindle secondaries, 4 Golgi tendon organs, 5 knee joint). Of the 18 hair receptors, 13 had stable activity patterns during walking, although 7 of the 13 had receptive fields not contacted by any object during walking. Units responsive to light touch were usually active when their fields were directly contacted, but in two of four units exhibiting skin-stretch sensitivity, events not associated with contact dominated the firing patterns. About 30 to 40 percent of all units identified under anesthesia were either inactive or unseparable from other units and noise during walking. Units active during walking are almost always recorded during the identification process but may defy attempts at physiological identification even with extensive dissection.

We have been impressed by the complexity and individuality of the firing patterns of most receptors studied. Many of these patterns would have been difficult to predict despite the availability of extensive information on receptor properties obtained by short-term studies. In extrapolating from receptor responses to artificial stimuli applied to immobilized animals, one is handicapped by the inability to predict accurately the occurrence of the effective stimuli during normal movements. The method we describe should be useful in determining the conditions during which a given afferent could contribute to modulation of motor output. It remains to be determined whether the special information content apparent in each unit's firing pattern is "used" by the central nervous system.

Long-term recording from peripheral afferents may also find clinical application in the new field of neural prosthesis. Recent progress in restoring function to limbs paralyzed by spinal cord injuries has demonstrated the feasibility of electrically stimulating existing musculature (18). As unit stability and electrode longevity are improved, devices for functional neuromuscular stimulation should benefit from the application of sensory feedback, which may be obtainable from intact skin and proprioceptors according to this technique.

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7. Electrode impedance is 100 to 250 kilohms and is stable for more than 2 months. Thermal, amplifier, and biological noise is usually below 20 μ V peak to peak, and action potentials are typically 50 to 400 μ V. Electrodes correctly inserted into the DRG proper almost always record separable units unless and until the insulation deteriorates.
8. A 90 percent platinum and 10 percent iridium alloy with Pyre-TML (polyimide) insulation (California Fine Wire Company, Grover City, Calif.) is stiff enough to penetrate the DRG but flexible enough to yield with normal movements.
9. Floating cut wires were originally used in the brain by J. Olds, J. F. Disterholt, M. Segal, C. L. Kornblith, and R. Hirsh [*J. Neurophysiol.* **35**, 202 (1972)] and refined for the cerebral cortex by M. Salzman and M. J. Bak [*IEEE Trans. Biomed. Eng.* **20**, 253 (1973)].
10. H. Burton and J. J. McFarlane, *J. Comp. Neurol.* **149**, 215 (1973).
11. The animal does not object to having the saddle manipulated (for example, while cables are

being attached) and is readily discouraged from chewing anchor sutures when bitter apple is spread on them.

12. The unipolar axon approaches the soma via a long, tortuous, and unmyelinated glomerular segment, which gives unit recordings distinctive polyphasic signatures.
13. Probably type G2. [P. R. Burgess, D. Petit, R. M. Warren, *J. Neurophysiol.* **31**, 833 (1968)].
14. We hypothesize that this unit was sensitive to hair movements or skin stretch generated during active toe movements prior to foot placement. Similar but less consistent midswing activation has been seen in an SAII light-touch receptor (13) in a toepad, where it may have been related to claw extension. Pauses after footfall may reflect rapid adaptation to a constant deformation typical of hair receptors.
15. P. Grigg and B. J. Greenspan, *J. Neurophysiol.* **40**, 1 (1977).
16. It is anatomically possible although statistically unlikely that this unit was a popliteus muscle spindle secondary [P. R. Burgess and F. J. Clark, *J. Physiol. (London)* **203**, 317 (1969)].
17. The time course of the rate of firing was similar to the time course of vertical force recorded during walking by J. T. Manter [*J. Exp. Biol.* **15**, 522 (1938)]. A similar firing pattern was seen in a Golgi tendon organ (GTO) from triceps surae, except that the GTO began to fire during late swing extension before load bearing began.
18. P. H. Peckham and J. T. Mortimer, in *Functional Electrical Stimulation: Applications in Neural Prostheses*, F. T. Hambrecht and J. B. Reswick, Eds. (Dekker, New York, in press).
19. The transducer was a saline-filled length of Silastic tubing stretched between tibial ridge and calcaneus with platinum electrodes at each end [modified from V. J. Prochazka, R. A. Westerman, S. P. Ziccone, *Electroencephalogr. Clin. Neurophysiol.* **37**, 649 (1974)].
20. We are grateful for the advice and criticisms of K. Frank, R. E. Burke, and W. B. Marks and technical assistance of G. Dold and B. Weston.

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Electrochemical Growth of Organic Charge-Transfer Complexes

There has been a surge of interest in the properties of highly conducting inorganic and organic salts. In many studies high-quality single crystal samples are a prerequisite for meaningful experiments. Single crystals are generally produced either by evaporation from solution or by solution diffusion techniques. In a recent report (1) Miller showed that some highly conducting inorganic crystals such as $K_2Pt(CN)_4X_{0.3} \cdot 3H_2O$ (where $X = Cl$ or Br) can be grown directly on a needle electrode by electrolysis.

This same method of electrolysis was used earlier to prepare some organic charge-transfer complexes (2). These crystals are produced by the electrolysis of a tetrahydrofuran solution of pyrene and the supporting electrolyte tetrabutylammonium perchlorate. The process consists of the oxidation of the parent hydrocarbon at a platinum electrode followed by the immediate interaction of this cation with the anion of the supporting electrolyte, giving rise to crystal formation. The crystals appear as black needles which can grow up to 3 mm long. In the particular case mentioned above, stoichiometric analysis showed the crystals to be pyrene₂perchlorate, but, if the electrolysis parameters are changed, pyrene perchlorate can be produced. Crys-

tal growth is very rapid initially; in less than 1 second very fine crystals (up to 3 mm long) form and then fall away from the electrode as a result of the movement of the liquid. This rapid growth shows that the crystals are at least as conductive as the solution; they have a very low resistance. A thicker, slower growth then follows from which single crystals can be harvested. This growth sequence has been recorded on film (3).

The technique of electrolytic growth of single crystals has a far wider application than just inorganic materials. With organic systems it is possible to prepare high-quality single crystals of complexes which seemingly cannot be prepared by the more well-known methods. The importance of this feature in the light of the current interest in such conducting organics as the tetracyanoquinodimethane (TCNQ) salts is obvious.

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