Voluntary Nerve Signals from Severed Mammalian Nerves: Long-Term Recordings

Abstract. *An electrode unit capable of detecting voluntarily elicited nerve signals for prolonged periods of time has been developed and tested. The electrode unit has been implanted around the distal ends of severed sciatic and peroneal nerves in rabbits. This electrode may prove suitable for implantation in human amputees for the purpose of recording neural control signals to activate prostheses with many degrees of freedom.*

An electrode capable of recording nerve signals for prolonged periods of time has attractive applications. Such an electrode offers the possibility of studying the voluntarily elicited nerve signals that are associated with muscular contractions and the resulting body movements. To date, this association has been restricted to myoelectric signals. Damaged or severed nervous tissue with poor regeneration properties could be patched by long-term use of an implantable recording electrode, together with electronic equipment and an implantable stimulating electrode. It has been shown that functionally related nerve fibers in a nerve trunk can be selectively stimulated from the surface of a nerve (*1*). Conversely, Lichtenberg and De Luca (*2*) have performed experiments indicating that signal patterns recorded from the surface of the nerves can be associated with a functionally distinct group of nerve fibers in the nerve. If the peripheral nerve continues to propagate signals, a suitable nerve-electrode recording interface is the major remaining obstacle. Another interesting application is to use the nerve signals for directly controlling electrically driven prosthetic devices. The present capability to fabricate prostheses with many degrees of freedom (*3–5*) indicates the suitability of neural control (*6*).

Previous attempts at constructing an electrode for recording nerve signals have been reported. Alter (*7*) wound platinum wires around peripheral nerves and recorded bioelectric signals during a short-term experiment. However, this arrangement was not suitable for long-term recordings. Marks (*8*) suggested that a nerve-regeneration electrode might be made by inducing cut nerve fibers to regenerate into a multiple microelectrode consisting of a Teflon structure with small metal-lined holes. Recently, a more refined technique (*9*) has proved the viability of the concept in amphibians. However, this approach may be difficult to apply to mammalian nerves because of their limited regenerative capability. The nerve regeneration electrode is by its nature highly selective in recording action potential trains of individual motoneurons. In general, several channels of information would be necessary to correlate the nerve signal with the associated muscle contraction, which is modulated by motoneuron recruitment as well as firing rate.

The cuff-type electrode configuration described in this report was specifically designed for recording functionally distinct nerve signals from the surface of severed nerves (*6, 10, 11*). The electrode unit and implantation procedure are applicable to human amputees. Several other configurations were considered; however, they were discarded because they could not be readily placed around nerves in amputees, especially in high-level amputees where the length of the remaining peripheral nerve is short. Recently, other investigators (*12–14*) have reported successful implantation of a cuff-type electrode around nerves that remained connected to their target muscle in mammalian limbs.

Several prototypes of our electrode have been implanted for periods up to 15 months. The biocompatibility of the proposed configuration was studied by histological analysis and electrical stimulation techniques. These results are the subject of an article presently being prepared.

The electrode unit consists essentially of a cloth tube containing two pairs of recording contacts (one pair inside and the other outside the tube), a cable, and a transcutaneous device. The design and construction are shown in Fig. 1. The electrode

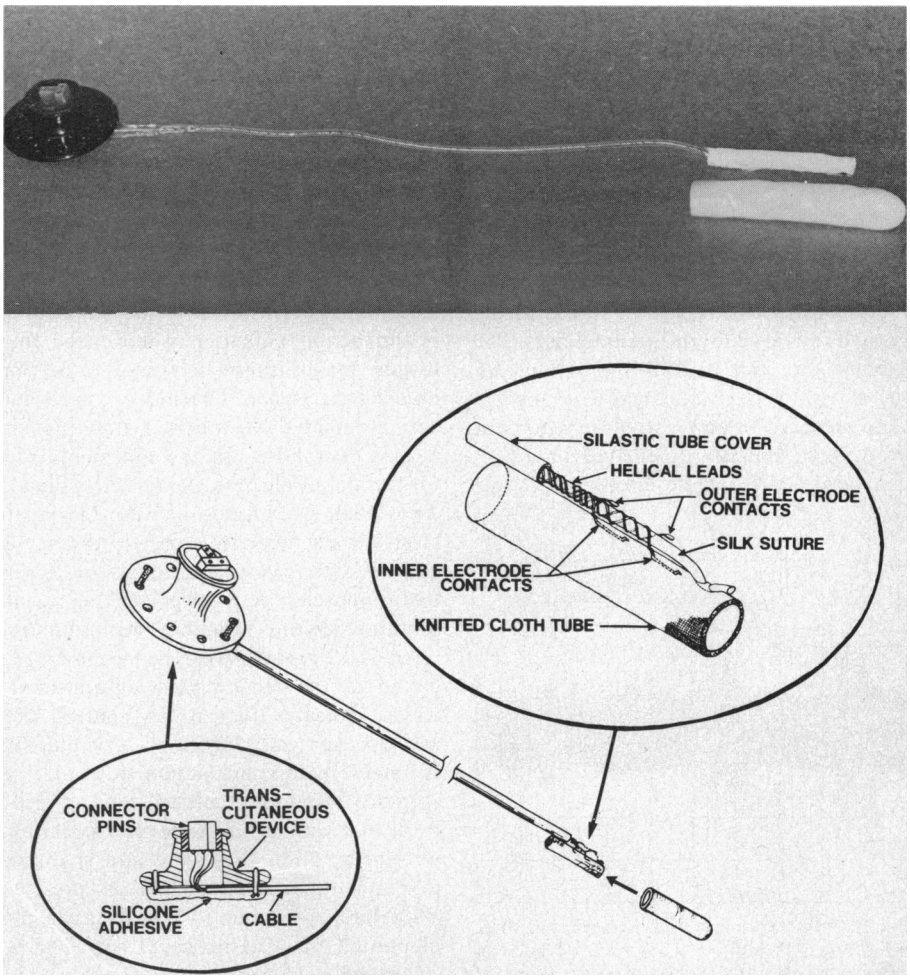

Fig. 1. An actual and a schematic view of the electrode unit implanted around severed nerves. The electrode tube is constructed from a patch of knitted Teflon or Dacron fabric. The mating edges are bonded together with Silastic adhesive. The length of the electrode tube is nominally 2.5 cm and the inside diameter is 1.4 times the diameter of the nerve to be enclosed. Four Teflon-insulated 90 percent platinum–10 percent iridium wires (0.27 mm in diameter) are woven into the tube fabric, terminating in two pairs of electrode contacts. One pair is located on the outer surface of the electrode tube and the other pair is located on the inner surface. The distal ends of the wires are stripped of insulation. The wires continue as a cable supported by a 2-0 surgical silk suture and enclosed in medical grade Silastic tubing. The cable terminates on connectors located in a Biocarbon device (Bentley Laboratories Inc.). The insulating cover for the electrode tube consists of a tube formed from Silastic 382 medical grade elastomer.

units were implanted by a sterile surgical procedure. Six electrode units have been implanted around the peroneal and sciatic nerves of anesthetized New Zealand white rabbits. An incision was made in the popliteal fossa and the appropriate nerve was cut as distally as possible. The nerve was freed from the connective tissue binding it in place. A silk suture was placed through the epineurium of the distal portion of the cut end. The nerve was then gently pulled into the electrode tube by the attached suture and was secured in place by tying the suture to the end of the electrode tube. The Silastic insulating cover was fitted securely over the electrode tube. The nerve and the electrode unit were arranged in an unobtrusive position and the incision was sutured. The transcutaneous Biocarbon connector was installed subcutaneously through a small incision in the thigh.

A stimulating electrode was implanted around the sciatic nerve in the proximal section of the thigh so that a controlled stimulus could be applied to the nerve. The stimulating electrode consisted of 90 percent platinum–10 percent iridium wires woven in knitted Dacron fabric wrapped around the nerve.

The inner and outer electrode pairs of the recording electrode unit were connected to two differential preamplifiers. The transcutaneous Biocarbon button of the recording electrode unit served as the ground reference for the preamplifiers. The rabbits were able to walk (hop about) on the day of the operation, and nerve signals were recorded in phase with their forward propulsion. Voluntarily elicited nerve signals recorded from the internal electrode pair on the first day had an amplitude of ~ 30 μv peak to peak. The amplitude of the nerve signals increased to ~ 40 μv during the second day and subsequently decreased with implantation time. Experiments to describe the behavior and cause of the signal amplitude decrease will be reported elsewhere. Figure 2 shows the nerve activity recorded from the distal end of a severed sciatic nerve while the rabbit was walking 2 weeks after implantation. Each burst of activity is associated with a step taken by the rabbit. On an expanded time scale, some individual action potential pulses could be seen. Their time duration was 0.25 to 0.5 msec.

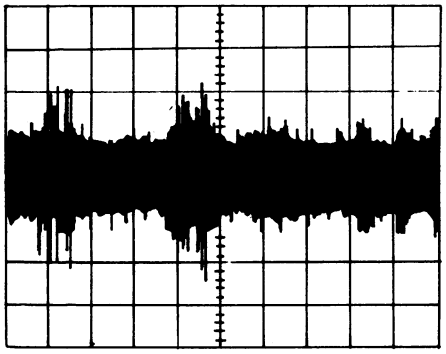

Fig. 2. Voluntary nerve activity recorded while the rabbit was walking. Each grouped burst of activity corresponds to a propulsion movement of the hind limbs. The first two distinct bursts were associated with pronounced "hops." The following, less distinct bursts were recorded while the rabbit was performing slight forward movements. The electrode unit had been implanted for 14 days. The time scale is 0.5 second per division and the amplitude scale 2 μv per division. This signal was filtered with a band-pass of 300 hertz to 3.7 khz. During the large bursts the peak-to-peak signal-to-noise ratio is 2.5.

Previous experiments with other electrode configurations indicated that a cuff-type electrode is susceptible to recording concurring myoelectric signals from adjacent muscles. A sequence of tests was performed to prove that the recorded signal originated from the enclosed nerve. An electrode unit similar to that shown in Fig. 1 was implanted around the distal end of a severed peroneal nerve in a rabbit. However, the Silastic cover was not placed over the tube of the electrode unit. The sciatic nerve was electrically stimulated by another implanted electrode. Figure 3, a and b, shows the signals recorded from the inner and outer electrode contacts, respectively. In these two traces the initial short (~ 0.5 msec) pulse corresponds to the nerve compound action potential, whereas the following longer pulse corresponds to the myoelectric signal. The nerve compound action potential has a higher amplitude in Fig. 3a than in Fig. 3b; the opposite is true for the amplitude of the myoelectric signal. This result is expected because the inner electrode contacts are closer to the nerve and the outer electrode contacts are closer to the muscles. A muscle blocking agent (decamethonium bromide) was administered to the rabbit. As can be seen in Fig. 3, c and d, the second pulse disappeared, which indicates that it was indeed the myoelectric signal. Hence, the first pulse is the nerve compound action potential. A stimulus response recorded from an electrode unit with a Silastic cover 2 weeks after implantation (Fig. 3, e and f) shows that no myoelectric signal is present. Therefore, at least up to 2 weeks after implantation the Silastic cover served as a successful insulator of the myoelectric signal.

Another indication that the signal presented in Fig. 2 is a nerve signal was obtained by computing its frequency spectrum. The signal had measurable frequency components from 0 to 5.5 khz. The frequency spectrum of the myoelectric signal from muscles of the rabbit's hind limb recorded with the described electrode had measurable frequency components from 0 to 800 hertz—a distinctly lower bandwidth.

The amplitude of the voluntarily elicited nerve signal decreases as a function of implantation time and appears to stabilize after 3 weeks. This result is compatible with histological observations, which indicate that the connective tissue growth between the nerve and the tube has stabilized, with only minor slow changes occurring in the

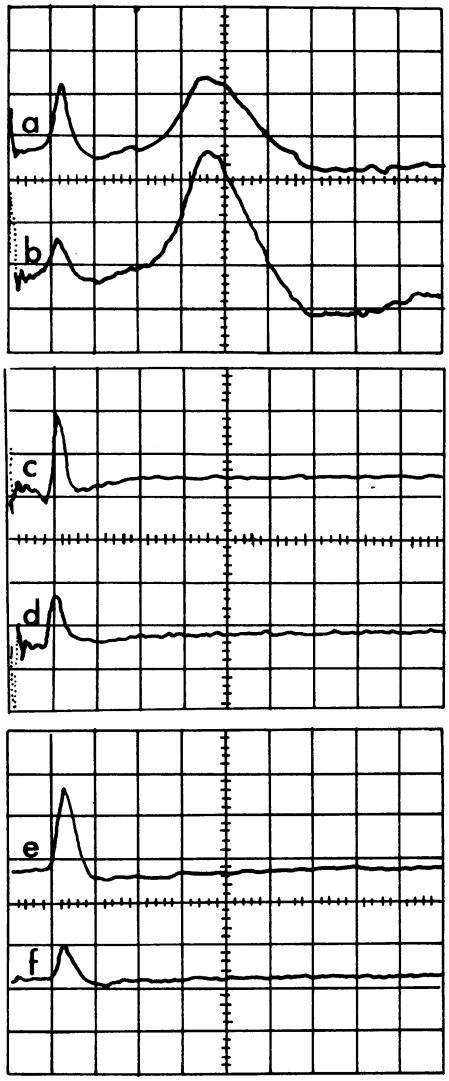

Fig. 3. Electrically stimulated responses recorded by the implanted electrode unit. The time scale is 0.5 msec per division and the amplitude scale is 100 μv per division. The top trace of each pair corresponds to the internal recording contacts and the bottom trace to the external contacts. The top pair was recorded with no insulating Silastic cover over the electrode tube. The middle pair was recorded when a muscle blocking agent was administered. The bottom pair was recorded when a Silastic cover was placed over the electrode tube. The Silastic cover effectively eliminates the myoelectric signal, represented by the pulse of longer duration in the top pair. The pulse of shorter duration appearing after a time interval of approximately 0.5 msec is the nerve compound action potential. The pulse at the beginning of the traces is the stimulation artifact.

organization of the connective tissue during the following 6 months. The presence of nerve signals after 3 weeks indicates that the motoneurons have not completely degenerated. A histological investigation to verify this point will be reported elsewhere.

After an implantation time of 3 weeks the connective tissue growth begins to provide a conductive pathway for the myoelectric signal. The Silastic cover can no longer effectively isolate the myoelectric signal. However, it is still possible to effectively reduce the myoelectric signal by subtracting the appropriately weighted signals from the internal and external electrode contacts. A larger myoelectric signal will be recorded from the external than from the internal contacts, most of the nerve signal will remain while the myoelectric signal is substantially reduced. Alternatively, it should be possible to remove most of the myoelectric signal recorded with the proposed electrode configuration by filtering the signal with a band-pass of 800 hertz to 5.5 khz.

This electrode unit was designed with the ultimate objective of implanting it in humans during an amputation. The proposed configuration allows several electrode pairs to be located around the perimeter of the tube so that two or more functionally distinct nerve signals can be recorded from one implanted electrode unit. This procedure has been successful in short-term experiments (*2*) with antidromic electrically stimulated nerves. The development of electrodes of this type provides exciting possibilities for neural control of prostheses with many degrees of freedom.

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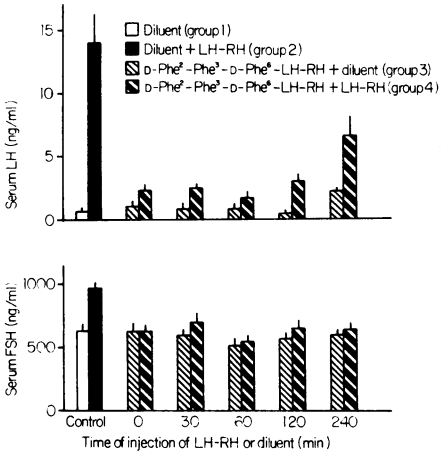
Blockade of Ovulation in Rats by Inhibitory Analogs of Luteinizing Hormone–Releasing Hormone

Abstract. *An antagonist of luteinizing hormone–releasing hormone (LH-RH), [D-Phe²-Phe³-D-Phe⁶]-LH-RH (Phe, phenylalanine), suppressed luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release in male rats in response to LH-RH for at least 4 hours. Three subcutaneous injections of 1 milligram of this antagonist into rats during proestrus completely suppressed ovulation, while a single injection of 1.5 milligrams per rat inhibited 95.3 percent of the preovulatory surge of LH, 84.2 percent of the FSH surge, and suppressed ovulation by 86.4 percent.*

Several in vivo and in vitro systems for measuring the antigonadotropin releasing activities of inhibitory analogs of luteinizing hormone–releasing hormone (LH-RH) have been described (*1, 2*). We have reported that [D-Phe²-D-Leu⁶]-LH-RH (Phe, phenylalanine; Leu, leucine), an inhibitory analog of LH-RH, suppressed the preovulatory surge of luteinizing hormone (LH) in proestrous hamsters and partially blocked ovulation (*3*). We also synthesized more potent antagonists of LH-RH, as judged by their powerful and prolonged suppression of LH and follicle-stimulating hormone (FSH) release in immature male rats (*4*). This report deals with blockade of the response to LH-RH in male rats as well as with suppression of the preovulatory surge of LH and ovulation in cycling rats by some of the most effective inhibitory analogs.

In the first experiment, the antigonadotropin releasing activity of [D-Phe²-Phe³-D-Phe⁶]-LH-RH was tested in vivo in immature male rats (Simonsen Laboratories), weighing 60 to 75 g (*1*). The animals were first injected subcutaneously with this analog (500 μg per rat) or with diluent alone (20 percent propylene glycol in saline). Simultaneously, or at different times thereafter, the rats were injected subcutaneously with 200 ng (per rat) of synthetic LH-RH or 0.2 ml of diluent. This quantity of LH-RH gave nearly maximum responses in immature male rats after careful dose-response studies. Animals were decapitated 30 minutes after the injection of LH-RH or diluent, and blood was collected for measurement of LH and FSH by radioimmunoassays. Serum samples were separated after centrifugation and stored at -20°C until assayed.



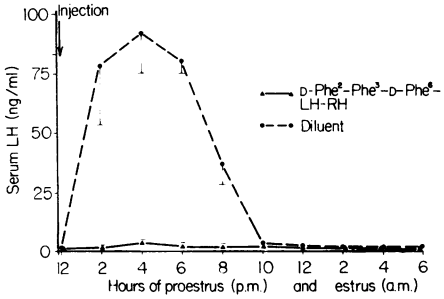


Fig. 1 (left). Time course in immature male rats of blockade of LH and FSH release in response to LH-RH by [D-Phe²-Phe³-D-Phe⁶]-LH-RH (500 μg per rat) injected subcutaneously. The values are expressed as means ± S.E. (standard error). The analog was injected at time 0. The rats were decapitated 30 minutes after administration of synthetic LH-RH (200 ng per rat) or diluent. Values for means ± S.E. for LH and FSH at 0, 30, 60, 120, and 240 minutes were significantly different from the values of group 2, respectively. Fig. 2 (right). Effect of a single subcutaneous administration of [D-Phe²-Phe³-D-Phe⁶]-LH-RH (1.5 mg) on the preovulatory surge of LH in proestrous rats. The differences in LH levels between animals treated with diluent (20 percent propylene glycol in saline) and analog were significant at 1400, 1600, 1800, and 2000 hours ($P < .01$).