A stimulating nerve cuff for chronic in vivo measurements of torque produced about the ankle in the mouse

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Warren, Gordon L., Christopher P. Ingalls, and R. B. Armstrong. A stimulating nerve cuff for chronic in vivo measurements of torque produced about the ankle in the mouse. J. Appl. Physiol. 84(6): 2171–2176, 1998.—Specific muscle training and chronic contractile measurements are difficult in rodents, especially in the mouse. The primary reason for this is the lack of a means for stimulating the motor nerve that does not damage the nerve and that permits reproducible measurements of contractility. In this paper, we describe procedures for the construction and implantation of a stimulating nerve cuff for use on the mouse common peroneal nerve. We demonstrate that nerve cuff implantation success rates can be high (i.e., 75–93%), as determined from measurements of maximal isometric torque produced by the anterior crural muscles. Isometric torque production is not adversely affected by the nerve cuff because the torque produced matches that observed in our established percutaneous stimulation model. We also demonstrate that use of the nerve cuff for stimulation is compatible with electromyographic measurements made on the tibialis anterior muscle, with no sign of stimulation artifact in the electromyographic signal.

Muscle contraction; electrical stimulation; electromyography

THE ROdent MODEL is the dominant model used in the study of skeletal muscle contractile function. In a Medline search of the period 1966–1997, rats and mice were employed in 43% of the skeletal muscle contractility studies, a percent >2.5-fold greater than that for any other single species. The main advantage of this model is the ability to measure contractile function in vivo, in situ, and/or in vitro. However, specific muscle training and chronic measurements of contractile function have been difficult in rodents. For example, it is difficult in rodents to study the functional adaptations to eccentric contraction-induced injury by using the more powerful within-animal study designs.

Direct electrical stimulation of the muscle has been used for muscle training and chronic contractile measurements (e.g., Refs. 2 and 9), but it is uncertain whether activation is equal throughout the muscle volume, and there is a good possibility of damage to the muscle fibers induced physically or electrochemically by the electrodes. Direct nerve stimulation would seem to be the best means of stimulation for chronic contractile measurements, but the construction and implantation of electrodes have been difficult in the rodent model. Relatively large, bulky electrodes (1, 6, 7) are hard to implant, especially in the mouse. These designs, coupled with inflexible lead wires, commonly result in nerve damage (Ref. 6; unpublished observations).

We have developed a relatively simple nerve cuff design for stimulation of the mouse common peroneal or tibial nerves. This design permits chronic in vivo contractile function measurements of the anterior and/or posterior crural muscles as well as training of these muscles and the tibial bone. The design should be easily adaptable to the rat model. In the following sections, the construction and implantation of the nerve cuff on the mouse common peroneal nerve will be described in detail, along with a discussion of the contractile function following surgery, success rates, and a comparison of contractile function with our established percutaneous stimulation model.

METHODS

Animals

Female ICR mice purchased from Harlan Laboratories were used. The mice were 3–4 mo old and weighed 34.3 ± 4.2 (SD) g at the time of surgery. They were housed with a 12:12-h light-dark photoperiod at the American Association of Laboratory Animal Care-accredited Laboratory Animal Resources facility at Texas A&M University. Before surgery, mice were anesthetized with either pentobarbital sodium (100 mg/kg) or a combination of fentanyl (0.33 mg/kg), droperidol (16.7 mg/kg), and diazepam (5 mg/kg). The latter anesthetic regimen was used whenever contractile measurements were to be made because of the depressive effect of pentobarbital sodium on in vivo contractility (4). All animal care and use procedures met the guidelines set by the American Physiological Society and were approved by the institutional Animal Care and Use Committee.

Experimental Procedures

Nerve cuff construction. The wire used most often in construction of the nerve cuff was Teflon-coated, multi-stranded 90% Pt-10% Ir wire (0.15-mm diameter; 10Ir/49T, Medwire-Sigmund Cohn). This wire is highly flexible and is deemed critical to the success of the nerve cuff design. We also found 36-gauge, Teflon-coated, multitinned Pd wire (AS 787–36, Cooner Wire) provides good results at a lower cost, but supply of this wire from the manufacturer was uncertain. Silver wire was found unacceptable from an electrochemical standpoint, and we were unable to find small-diameter multitinned stainless steel wire with the flexibility of the Pt-Ir or Pd wire. The main disadvantage of the Pt-Ir-based nerve cuff is its cost (i.e., $5.40 to $10.00 (US dollars) per nerve cuff depending on whether the wire is bought in bulk).
Two lengths of wire are cut (i.e., 7.8 and 7.9 cm), and the ends are deinsulated by flame. The distal ends of the wires (i.e., those to be in contact with the nerve) are deinsulated by 2.5 mm while the other (i.e., proximal) wire ends are deinsulated by ~5 mm. This step and all subsequent steps in the construction process are done under a dissecting microscope. The tips of the wire ends are then glued with epoxy to prevent unraveling of the strands. The wires are tied together side by side in a staggered manner so that the longitudinal spacing between the two distal ends is 1.5 mm (Fig. 1). Three 15.2-cm-long 6-0 silk sutures are used to make these ties, with the spacing between knots as shown in Fig. 1; the excess suture of the middle knot is cut off. The knots and the portion of the wires between the knots are then glued with epoxy.

The 2.5-mm uninsulated portions of the wires are then bent at right angles to the length of the wires (Fig. 2). Each bend occurs at the end of the insulated wire portion, and the bend is directed away from the side of the nerve cuff with the suture knots. This and subsequent manipulations of the wires are made by using two pairs of serrated micro-dissecting forceps: one pair holds the insulated wire just adjacent to the uninsulated portion, and the other pair is used to elicit the desired bend. The uninsulated wire portions are then bent around a 21-gauge needle held in a clamp to form the loops as shown in Fig. 2. The opening left in each wire loop is just enough for the nerve to pass through. It is important that several very small bends be placed in the uninsulated wire portion before the loop is finally shaped. This prevents collapse of the loop on the nerve as the loop is closed during the implantation procedure. Before implantation, the outside portions of the wire loops (i.e., that portion of the wire not in contact with the nerve) are heavily coated with silicone adhesive (Silastic medical adhesive: silicone type A, Dow Corning). This is done to help stabilize the loops and to minimize stimulation of adjacent tissues.

The final step in constructing the nerve cuff is the construction of the protective cover for the proximal end of the nerve cuff wires (i.e., the wire ends connected to the stimulator) (Fig. 1). A 1-cm-long segment of an 18-gauge needle is needed along with a 1-cm length of polyethylene tubing (PE-200; 1.4 mm ID, 1.9 mm OD). Sharp edges on the needle segment are removed externally with sandpaper and internally by passing a 25-gauge needle through the needle segment. During implantation, the proximal wire ends of the nerve cuff are passed through the needle segment and then folded back onto the outside of the needle segment. The polyethylene tubing is then passed over the needle segment, firmly sandwiching the wires between the needle segment and the tubing (Fig. 1).

Nerve cuff implantation. Before implantation, all components of the nerve cuff with the exception of the polyethylene tubing are autoclaved; gas sterilization is recommended for the polyethylene tubing. The nerve cuff implantation is conducted by using aseptic technique. The hair covering the dorsal cervical region and the lateral portion of the hindlimb is clipped, and the remaining hair removed by using a depilatory. The exposed skin is then aseptically prepared. For implantation of the nerve cuff on the left common peroneal nerve, the mouse is placed in right lateral recumbency. The left hindlimb is positioned so that the femur (which is visible through the skin) is perpendicular to the cranial-caudal axis of the mouse. The lower left leg is in turn held so that the tibia is perpendicular to the femur (Fig. 3). This positioning is maintained by taping the left foot on top of the right foot and both feet in turn to the underlying surgical table.

A 1.5-cm-long incision is made through the skin as indicated in Fig. 3. A 5- to 6-mm incision is then made in the

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**Fig. 1.** Construction of the nerve cuff: alignment of wires and suture knots before formation of wire loops. Protective cover on proximal end of wires is also shown.
biceps femoris muscle parallel to the femur, offset in the posterior direction by ~2 mm (Figs. 3 and 4). This incision parallels the muscle fiber longitudinal orientation and stops distally at the insertion site of the biceps femoris muscle into the tibial head. The incision is then continued but in a direction parallel to the tibial long axis, offset ~1 mm proximal to the insertion of the biceps femoris tendinous sheath into the tibia (Figs. 3 and 4). This extension of the incision is 3–4 mm long and should expose the common peroneal nerve. We prefer to make this cut with microdissecting scissors because connective tissue holding the biceps femoris muscle to the underlying triceps surae muscles will also have to be cut.

The flap of muscle created by the two incisions is folded back, exposing the common peroneal nerve. With use of blunt-tip microdissecting forceps and a fine-tip glass rod, the common peroneal nerve is freed from the biceps femoris muscle and the connective tissue just proximal to the nerve passing over the lateral gastrocnemius muscle. At least 2 mm of nerve must be freed for nerve cuff implantation.

A 10-cm-long metal tube (2 mm OD) is passed subcutaneously from the proximal end of the skin incision and is pushed through an ~1-mm nick in the dorsal cervical skin. The proximal end of the nerve cuff is fed through the tube in a distal-to-proximal direction, and the tube is then pulled through from the dorsal cervical end. As the tube exits the skin hole in the dorsal cervical region, the proximal ends of the nerve cuff are externalized. The nerve cuff is positioned so that the most proximal knot on the nerve cuff is at the proximal end of the incision through the biceps femoris muscle (Fig. 4). Small (i.e., 5–10°) bends are then made in the nerve cuff to ensure that the distal end of the nerve cuff parallels the nerve; the openings of the two wire loops should face up. With the two wire loops of the nerve cuff held slightly posterior and lower than the nerve, the freed portion of the nerve is lifted through the openings of the wire loops by using the fine-tip glass rod. If possible, the loops are closed off by using forceps, thus locking the nerve in the cuff. We have found this step to be more critical for the tibial nerve than for the common peroneal nerve.
the common peroneal nerve because the flap of biceps femoris muscle effectively seals off the open end of the wire loops. The suture attached to the most distal knot on the nerve cuff is used to tie the nerve cuff to the proximal tendinous sheath of the lateral gastrocnemius muscle adjacent to the nerve; this tie off is loosely applied and is mainly designed to keep the longitudinal orientation of the nerve cuff aligned with that of the nerve. The flap of biceps femoris muscle is pulled over the nerve cuff and sutured into place by using 6-0 silk suture. Ideally, the most proximal suture knot on the nerve cuff should now just be barely visible from underneath the muscle flap. The two suture ends extending from the knot are passed through adjacent muscle tissue and the two suture ends are tied together, thus securely locking the nerve cuff in place. The skin incision is then closed by using 6-0 silk suture.

The access site to the nerve cuff in the dorsal cervical region is prepared depending on how frequently access is needed to the nerve cuff. If access is required only every few days or more, we prefer to use a 9-mm wound clip to close the skin, leaving the protected end of the nerve cuff immediately beneath the wound clip. If daily access is required, use of a Velcro patch as described by White-Welkley et al. (8) is recommended. A 1.7-cm-diameter circle is cut from a strip of Velcro. A small hole is cut through the "loop" side of the Velcro patch, and the wires from the nerve cuff are passed through the hole. The loop side of the Velcro patch is then glued (Medbond cyanoacrylate adhesive) and sutured to the skin so that the hole in the patch is centered over the hole in the skin. The "hook" side of the Velcro patch is placed on the loop side, sandwiching the protected end of the nerve cuff between the two layers. Rats appear to tolerate the Velcro patch better than do mice.

Immediately after surgery, the viability of the nerve cuff is checked by determining the stimulation voltage necessary to elicit ankle dorsiflexion while the muscles are under no external load. The number of stimulations should be kept to a minimum (i.e., <5); otherwise, problems associated with nerve cuff movement may arise.

Recovery from the surgical procedure is rapid. Ambulation appears normal immediately after surgery in most mice. In the days after surgery, the skin closure sutures must be monitored. If the mouse extracts the sutures before the skin incision has healed, cyanoacrylate adhesive is applied after resuturing of the skin wound. No infection has been observed at either the implantation or the externalization sites, although care must be taken to minimize trauma to skin around the externalization site.

Torque measurement. Torque production by the anterior crural muscles of the mouse was measured by using the apparatus previously described (e.g., Refs. 4 and 5). Nerve stimulation was done by using 75-µs biphasic pulses produced by a Grass S48 stimulator with a SIU-5 stimulus isolation unit set to capacity coupling mode. Isometric tetanic stimulations were elicited by 200-ms trains of pulses at 300 Hz.

Electromyography (EMG) of the tibialis anterior (TA) muscle. To test the compatibility of common peroneal nerve stimulation using our nerve cuff design with TA muscle EMG measurement, we chronically implanted EMG electrodes just beneath the fascial sheath covering the TA muscle (n = 12.
mice). The electrodes consisted of 8.9-cm-long lengths of the same wire used for the nerve cuffs (i.e., Pt-Ir wire). The recording surfaces were prepared by deinsulating 3.0 mm of the wires' distal ends. The wires were routed underneath the fascial sheath at the TA muscle's midbelly by using a 23-gauge needle. The two wires ran parallel to each other at a spacing of 2.0 mm and were placed so that their longitudinal axes were perpendicular to that of the superficial TA muscle fibers. The wires were held in place by being sutured to the distal tendinous sheath of the biceps femoris muscle and by application of a small dab of Medbond cyanoacrylate adhesive to their distal tips. The proximal ends of the wires were routed to the dorsal cervical region where they were connected to a Grass P-15 amplifier. A wire acutely implanted beneath the skin in the abdominal region served as the reference electrode. The EMG signal was band-pass filtered (i.e., 10–3,000 Hz) and subsequently sampled by the same computer used for the torque measurements. EMG measurements were made on a given animal no earlier than 5 days after implantation of the EMG electrodes and 16 days after implantation of the nerve cuff.

RESULTS AND DISCUSSION

Nerve cuffs were implanted on the common peroneal nerve in 112 mice. Criteria for a successful implantation were established from data collected by using our in vivo model with percutaneous stimulation and fentanyl-droperidol-diazepam anesthesia (Refs. 3, 4; unpublished observations); mean maximal isometric torque of the anterior crural muscles in those experiments (n = 300 mice) equaled 3.17 ± 0.42 N·mm, with 95% of the values being >2.54 N·mm. In the present study, we considered a nerve cuff implantation to be successful if torque equaled or exceeded 2.54 N·mm. In three-quarters of the experiments, torque was checked for the first time between 10 and 26 days after surgery. If the nerve had been damaged, complete recovery in torque was not evident until ~60 days after surgery. Generally, it was easy to distinguish a “successful” implantation from one that was not; torque averaged 2.9-fold higher in successful mice.

Overall, 75% of the implantations were successful; this percent includes implantations conducted during all stages of the technique development. More recently, the success rate has been 93% (i.e., 39 of 42). This improvement is attributed to 1) leaving a longer portion of the nerve cuff beneath the biceps femoris muscle flap, thus reducing the torque applied to the nerve by the cuff; 2) freeing up more nerve before placing in the nerve cuff; and 3) ensuring that the wire loops do not collapse on the nerve.

The mean maximal isometric torque of all successful mice equaled 3.38 ± 0.50 N·mm, 7% greater than the mean of the data for percutaneous stimulation (i.e., 3.17 N·mm); however, body mass of the mice used in the present study was on average 9% greater. These data indicate that successful nerve cuff implantation does not adversely affect the force-producing capability of the anterior crural muscles.

The allowance of sufficient recovery from the nerve cuff implantation is critical to the success of the procedure. If torque was measured before 5 days after the
surgery, then erratic torque values were observed, presumably because connective tissue had not developed sufficiently to fix the nerve cuff in place. The erratic results were attributed to movement of the nerve cuff wire loops relative to the nerve, resulting in some stimulation of the adjacent gastrocnemius muscle. However, if torque was evaluated after 7 days, we observed good reliability for the measurement of isometric torque when using this stimulation technique; the within-subjects SD of replicate determinations measured six times over 2 mo equaled 5.5% (n = 6 mice). Furthermore, the nerve cuffs are easily viable out to 4 mo after surgery (n = 6 mice), and we have even followed two mice for 7 mo. When the nerve cuff does fail at these later times, it is usually due to damage of the nerve cuff proximal end inflicted by the mouse or breakage of the nerve cuff wires in the hip region.

We have found the nerve cuff stimulation procedure to be compatible with EMG measurements made on the TA muscle. We see no evidence for stimulation artifact in the EMG signal. Figure 5A shows a representative plot of EMG root mean square as a function of stimulation voltage. Above 1.4 V, there is no further increase in the EMG root mean square as one would expect for an artifact-free signal. The biceps femoris and triceps surae muscles effectively insulate the nerve cuff from the EMG electrodes. The relationship between EMG root mean square and isometric torque production is illustrated in Fig. 5B. Figure 6 shows the stability of both EMG root mean square and isometric torque over the 2-wk period after performance of 150 concentric contractions (n = 8 mice).

In conclusion, we have demonstrated a means for chronic stimulation of the common peroneal nerve in the mouse by using a simple electrode design and implantation procedure. Success rates are high. Training and/or chronic contractile measurements can be initiated by ~2 wk after surgery with assurance that contractile function is stable. We established a minimum isometric torque criterion for categorizing an implantation as successful or not. There was a clear dichotomy in torque production between mice with damaged and undamaged nerves as long as the assessment was made in the first month (i.e., before recovery of damaged nerves had occurred). Finally, with minor modifications, this nerve cuff design could be adapted for use in other species.

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