Published in "Journal of Neuroscience Methods, 2010
doi: 10.1016/j.jneumeth.2010.03.011"
which should be cited to refer to this work.

Methods for chronic recording of EMG activity from large numbers of hindlimb muscles in awake rhesus macaques

Heather M. Hudson[a,1], Darcy M. Griffin[a], Abderraouf Belhaj-Saïf[b], Sang-Pil Lee[a,2], Paul D. Cheney[a,*]

[a] Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160, United States
[b] Unit of Physiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland

Studies of the neural control of movement often rely on the ability to record EMG activity during natural behavioral tasks over long periods of time. Increasing the number of recorded muscles and the time over which recordings are made allows more rigorous answers to many questions related to the descending control of motor output. Chronic recording of EMG activity from multiple hindlimb muscles has been reported in the cat but few studies have been done in non-human primates. This paper describes two chronic EMG implant methods that are minimally invasive, relatively non-traumatic and capable of recording from large numbers of hindlimb muscles simultaneously for periods of many months to years.

1. Introduction

In the field of motor control, chronic recording of EMG activity from large numbers of muscles is highly advantageous. Thus far, the hindlimb has been extensively studied in the cat. A wide range of EMG implant methods have been used, including patch electrodes sutured to muscle surfaces, wire electrodes implanted in muscles and wire electrodes tied or sutured to muscles. These EMG electrodes were tunneled subcutaneously to either back or head connectors. In each study, relatively few muscles were implanted and the length of study ranged from a few weeks to several months (Prochazka et al., 1977, 1989; Hoffer et al., 1987, 1989; Drew, 1988; Loeb, 1999; Bretzner and Drew, 2005). In non-human primates, several studies have described methods of implanting EMG electrodes in muscles of the forelimb (Fetz and Cheney, 1980; Wolpaw and Herchenroder, 1990; Miller et al., 1993; Belhaj-Saïf et al., 1996; Park et al., 2000). Studies involving chronic EMG implants in the hindlimb of non-human primates are fewer in number and involve a relatively small number of muscles (Recktenwald et al., 1999; Hodgson et al., 2001; Courtine et al., 2005). The goal of this study was to adapt an existing method developed in our laboratory for recording EMG activity from large numbers of chronically implanted forelimb muscles (Park et al., 2000) for use in the hindlimb. The two methods described in this study, the arm-mounted subcutaneous implant and the cranial-mounted subcutaneous implant, have yielded stable recording of EMG activity from 19 muscles of the monkey hindlimb for periods up to 31 months.

2. Materials and methods

All surgeries were performed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility using full aseptic procedures.

Park et al. (2000) described two EMG implant methods for chronic recording from 24 forelimb muscles simultaneously. We have expanded on these methods and adapted them for chronic recording from 19 muscles of the hindlimb simultaneously. Electrodes were placed both proximally and distally in soleus so the total number of EMG channels was 20. We used two different approaches: (1) an arm-mounted subcutaneous implant method (Fig. 3A) and (2) a cranial-mounted subcutaneous implant method (Fig. 3B). The results described are from one arm-mounted subcutaneous implant performed in one monkey (Monkey F) and one cranial-mounted subcutaneous implant performed in a different monkey (Monkey C). In both cases, 19 muscles of the hindlimb were implanted including hip muscles: gracilis (GRA), adductor brevis

* Corresponding author at: Department of Molecular and Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66160, United States. Tel.: +1 913 588 7404; fax: +1 913 588 7430.
E-mail addresses: hhudson2@kumc.edu (H.M. Hudson), dgriffin2@kumc.edu (D.M. Griffin), abderraouf.belhaj@unifr.ch (A. Belhaj-Saïf), slee5@kumc.edu (S.-P. Lee), pcheney@kumc.edu (P.D. Cheney).
[1] Tel.: +1 913 588 0005.
[2] Tel.: +1 913 588 9070.

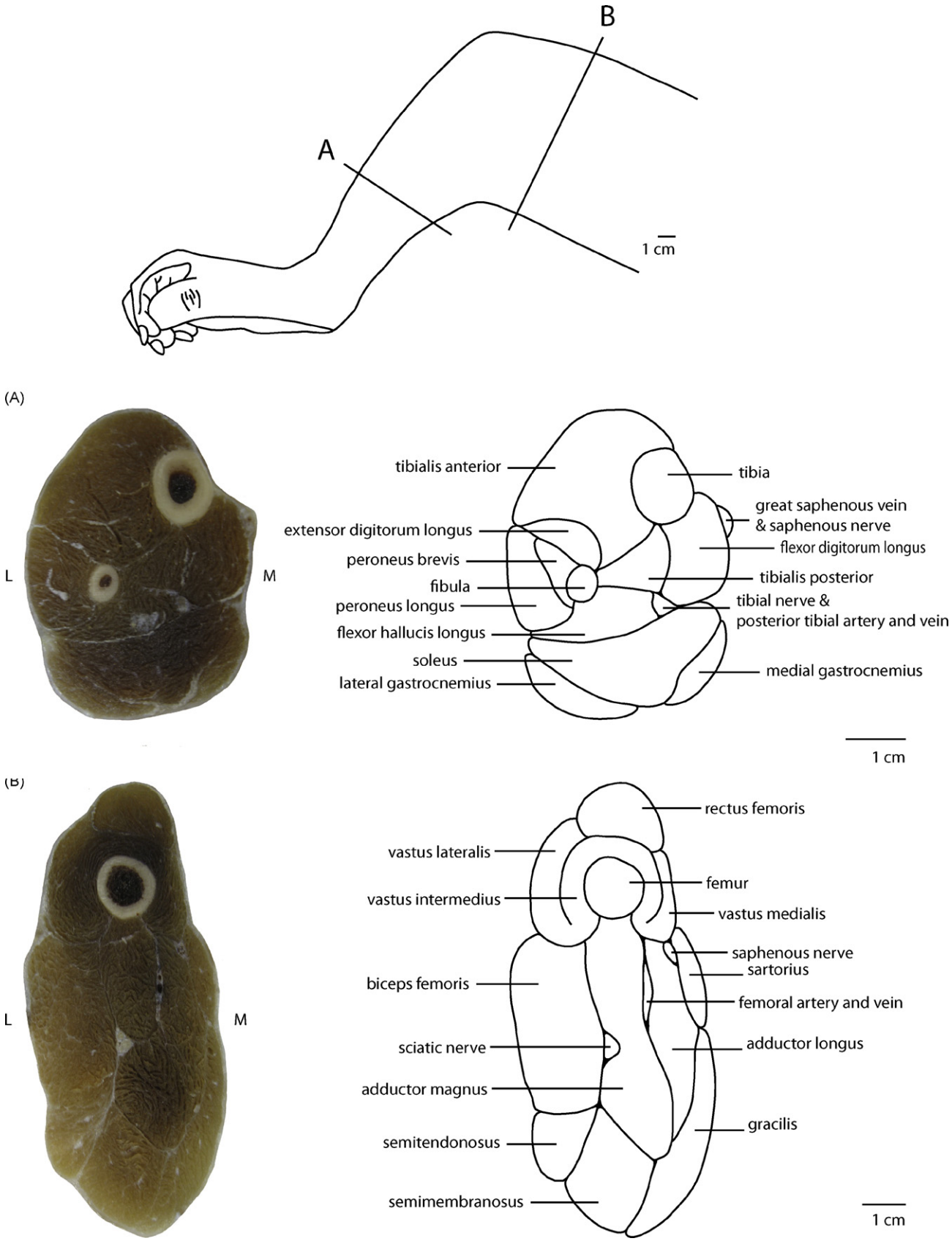

Fig. 1. Cross-section of muscle anatomy in the lower leg (A) and upper leg (B). A cadaver hindlimb was frozen and sectioned using a 19 tooth/inch, bi-metal band saw blade (L.S. Starret Co.). Photographs were taken of each cross-section and the boundaries between muscles were traced directly from the sections. Where separation of muscles was difficult to discern, macroscopic ultraviolet epifluorescence was used to identify fascial boundaries. Identification of muscles was confirmed by cross-referencing to a dissected cadaver limb with muscles intact. Medial (M) and lateral (L) aspects of the hindlimb are marked on the photographs in A and B.

(ADB), gluteus maximus (GMAX), and tensor fascia latae (TFL); knee muscles: biceps femoris (BFL), semimembranosus (SEM), semitendonosus (SET), rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM); ankle muscles: tibialis anterior (TA), peroneus longus (PERL), medial gastrocnemius (MG), lateral gastrocnemius (LG), proximal soleus (SOLp), and distal soleus (SOLd); digit muscles: flexor digitorum longus (FDL) and extensor digitorum longus (EDL); and intrinsic foot muscles: flexor hallucis brevis (FHB) and extensor digitorum brevis (EDB). Fig. 1 illustrates the position of each muscle in cross-sections of the proximal (upper leg) and distal (lower leg) hindlimb.

Fig. 2 demonstrates the use of T1- and T2-weighted MRIs in identification of muscle separation by fascial tissue. Magnetic resonance imaging (MRI) was performed using a Siemens Allegra 3T scanner with a custom built transmit/receive Helmholtz radio frequency (RF) coil consisting of two flexible surface loops with diameters of approximately 6 cm. T1-weighted images were acquired using magnetization prepared rapid acquisition of gradient echo (MPRAGE) sequence with parameters as follows: repetition time = 1500 ms, echo time = 2.09 ms, inversion time = 300 ms, slice thickness = 1 mm, field of view = 10 cm, matrix size = 192 × 192, flip angle = 8°, and number of average = 3. T2-weighted images were acquired using a fast spin echo sequence with the following parameters: repetition time = 3000 ms, echo time = 49 ms, slice thickness = 3 mm, field of view = 7 cm, matrix size = 256 × 256, echo train length = 5, and number of average = 2. While bone is easily identifiable with both types of MRI, separation of muscles by identifying fascial boundaries is clearer in the T1-weighted image. Most of the fascial boundaries evident in the section of the upper leg (Fig. 1A) are evident in the T1 MRI, although some are less clear. However, the T2 image (lower leg) was much less effective in revealing fascial planes between muscles, at least in this cadaver specimen.

2.1. Arm-mounted subcutaneous implant

2.1.1. Connectors

The arm-mounted subcutaneous implant uses single layer connector modules (ITT Canon pin, 031-9540-000; plastic Centi-Loc pin strip, 144-9614-060) that can be affixed to the skin as described in Park et al. (2000). Forty multi-stranded stainless steel wires (Cooner Wire, AS632) were cut to lengths appropriate for the 20 pairs of EMG wires to be implanted. The wires were divided into four modules: proximal one (ADB, TFL, BFL, RF, VL, VM), proximal two (GRA, GMAX, SEM, SET), distal one (MG, LG, SOLp, SOLd, FDL) and distal two (TA, PERL, EDL, FHB, EDB). Connectors were constructed as described in Park et al. (2000).

2.1.2. Surgical protocol

The monkey was treated with antibiotic (injectable liquid penicillin, 6000 U/kg) 1 day before surgery, 1 day post-surgery and 3 days post-surgery as a prophylactic measure against infection. The monkey was initially tranquilized with ketamine (10 mg/kg) and medetomidine (0.10 mg/kg) before induction of surgical level isoflurane gas anesthesia. Atropine (0.04 mg/kg) was given to reduce secretions and prevent bradycardia. The monkey's forelimb, neck, back, hip, hindlimb and foot were shaved and scrubbed (Betadine: 10% povidone–iodine). The monkey was placed on the surgery table on his left side and temperature, blood pressure and pulse monitors were applied. The monkey was draped except for the forelimb, back and hindlimb.

The connector modules were first laid on the lateral surface of the proximal forelimb. The tips of each wire were color-coded with permanent marker to ease the identification of each wire after tunneling. Although more costly, Cooner AS632 wire is available in different colors of insulation. Two small incisions (~1 cm) were made approximately half way between the shoulder and the elbow on the lateral surface of the proximal forelimb. These incisions functioned as entry points for the wires running subcutaneously to the hindlimb. A vertical incision (~4 cm) was made on the back near the midpoint between the shoulder blades. This incision functioned first as the exit point for the subcutaneous tunneling of wires from the forelimb to the hindlimb. A tissue pocket at this incision provided a space to keep extra wire lengths. These loops also served as anchoring points for the wires (arrow 2, Fig. 3A).

Custom designed needles, fabricated from stainless steel rods (Small Parts, 18-8 stainless steel: E-SWX-3035, E-SWX-3043) were used to tunnel the EMG wires. These flexible needles have diameters of 0.035 in. (~0.90 mm), 0.045 in. (~1.14 mm) and 0.06 in. (~1.52 mm) and lengths ranging from 7.5 to 42 cm. Each needle has a sharpened tip with a non-cutting edge and is flattened with 1–4 eyes on the other end. The edges of the eyelets were rounded to avoid possible damage to the EMG wire. The wires were threaded through the eyes and folded back for tunneling under the skin. To test the ability of the wire and insulation to withstand the stress of being bent back at the needle eyelet and then tunneled under the skin, we subjected wires to pulling forces after threading them through the eyelets of the tunneling needles. We found that the insulation could withstand forces sufficient to break the wire before becoming compromised. Even after the wire broke, the insulation stretched out but otherwise remained intact.

From the proximal forelimb (arrow 1, Fig. 3A), the four bundles of wires (1 per connector module) were tunneled separately to the back incision (arrow 2, Fig. 3A). A piece of surgical tape was then loosely wrapped around each bundle of wires and marked with a skin marking pen to identify the bundles later in surgery. Each bundle was then tunneled to a separate, small puncture incision (1–2 mm made with a #11 blade) in the skin above the hip (arrow 3, Fig. 3A). From the most lateral hip incision 1, a pair of wires was tunneled to a small puncture incision in the skin over GMAX. The remaining wires from the most lateral hip incision were tunneled to a small puncture incision on the lateral surface of the proximal hindlimb (arrow 4, Fig. 3A). From here, pairs of wires were tunneled to small puncture incisions in the skin above GRA, SEM and SET. Incision points were staggered to minimize cross-talk between adjacent muscles. In our experience, compared to the forelimb, cross-talk was less problematic. This is probably due to the greater distances between electrode pairs than for the forelimb. From the second hip incision, the bundle of wires was tunneled to a puncture incision in the skin on the lateral surface of the distal hindlimb. From here, pairs of wires were tunneled separately to small puncture incisions in the skin above MG, LG, SOLp, SOLd and FDL. From the third hip incision, the bundle of wires was tunneled to a puncture incision in the skin on the anterior surface of the distal hindlimb. From here, pairs of wires were tunneled separately to small puncture incisions in the skin over TA, PERL, EDL, FHB and EDB. From the most medial hip incision 4, two pairs of wires were tunneled to a puncture incision in the skin on the anterior surface of the proximal hindlimb. From here, one pair of wires was tunneled to a puncture incision in the skin above BFL. A second pair of wires was tunneled to a puncture incision in the skin above VL. A third pair of wires was tunneled from the medial hip incision to a small puncture incision in the skin above TFL. The remaining three pairs of wires were tunneled from hip incision 4 to a puncture incision on the medial surface of the proximal hindlimb. From here, individual pairs of wires were tunneled to small puncture incisions in the skin above RF, VM and ADB. Tunneling to a common puncture incision distal to the target and then tunneling back proximally to sites over individual target muscles helped anchor the EMG wires.

Each wire was pulled through, leaving 8–10 cm of exposed wire at the incision on the back (arrow 2, Fig. 3A). Each wire was cut to length, leaving 6–7 cm exteriorized at the target muscle site. For

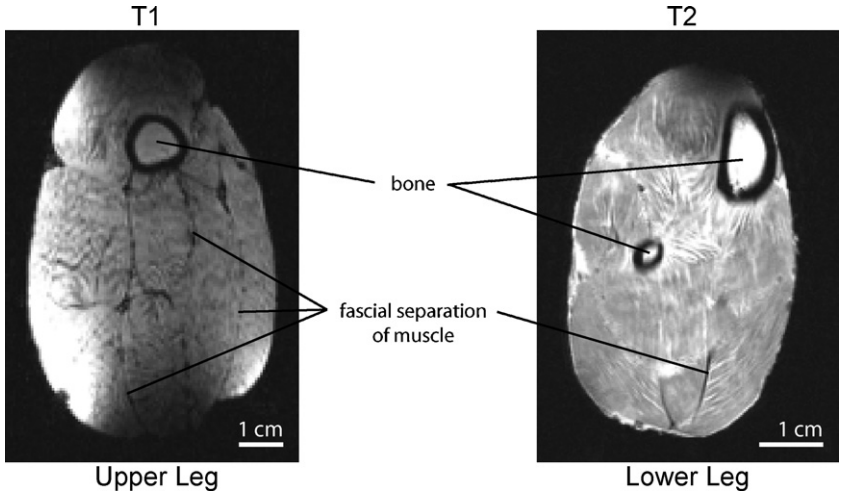

Fig. 2. T1- and T2-weighted MRIs of the upper and lower hindlimb in a cadaver rhesus monkey leg. Bone is easily identifiable with both types of imaging. Fascial boundaries separating muscles are more pronounced in the T1-weighted image. Bright spots on the periphery of the T1-weighted image are due to placement of the Helmholtz RF coil loop relative to the leg.

each wire, 2–3 mm of insulation was removed from the tip. Each wire was then back fed into a 22-gauge hypodermic needle (type D, beveled to minimize tissue damage) and folded back along the shaft of the needle. The wire was then inserted into the muscle in a proximal direction along the length of the muscle through the same puncture incision in the skin used for tunneling. The wire was then held at its entry point into the skin and the needle was removed leaving the EMG wire with a terminal hook embedded in the muscle belly. The two wires of a pair were inserted into the muscle with a separation of approximately 5 mm.

The wire insertion points for specific muscles were identified on the basis of external landmarks and palpation of muscle bellies. Landmarks and muscle locations were developed from dissection studies in *Macaca mulatta*. A dissected cadaver limb was also available for reference during surgery.

Proper placement of each electrode pair in a muscle was tested by stimulating through the electrodes with brief stimulus trains (biphasic pulse, 0.2 ms each phase, ~50 Hz) while observing the evoked movements. Palpating the tendons of implanted muscles during stimulation is another useful technique to confirm proper electrode placement. However, this method is useful only for muscles with easily identifiable tendons such as the ankle and foot muscles. Yet another method to help confirm proper location is to observe movement of the wires with passive movements at joints that will stretch and shorten the implanted muscle. This method is most useful for digit muscles but must be performed carefully with tunneled wires because the exposed loop of wire at the implant site is generally very short. If proper placement was not confirmed, the wires were removed and reinserted.

After implantation and confirmation of the proper location at each muscle, each pair of wires was isolated at the back incision (arrow 2, Fig. 3A) and pulled slowly, avoiding the formation of any kinks, until the wire at the implant site was completely subcutaneous. The hindlimb was then passively moved in all directions, which pulled additional lengths of EMG wire subcutaneously as the muscles lengthened. After all muscles were implanted, each bundle of wires leading to the arm connectors was pulled into the back incision, leaving only 3–4 cm of exposed wire at the connector module on the forelimb (arrow 1, Fig. 3A). At the incision site on the back, a small pocket was created using blunt dissection in the subcutaneous layer. The identifying surgical tape was removed from each bundle. Excess wire was formed into loops and placed in the subcutaneous pocket, thus eliminating all exposed wire. The loops of extra wire served to both accommodate movement of the limb and securely anchor the implant. The incision site was then flushed with saline followed by antibiotic (injectable liquid penicillin) and sutured (3-0 stainless steel). The puncture incisions at the implant sites were sealed with tissue glue (Henry Schein, Nexaband: 893-8109).

The four connectors were affixed to the monkey's proximal forelimb with an elastic medical adhesive tape (Johnson & Johnson Health Care System, Elastikon elastomeric tape: 5174, 2 in. width) as described in Park et al. (2000). To protect the implant, the monkey wore a custom fitted primate jacket (Lomir Biomedical, model PJ05) reinforced with stainless steel mesh (Whiting and Davis, SM5SS) while in its home cage. Implanting 20 pairs of EMG leads in hindlimb muscles with this method required approximately 9 h of surgery.

Following surgery, the monkey was closely monitored until it was fully awake and able to sit and stand without assistance. Postoperative analgesics (buprenorphine, 0.01 mg/kg) were given for 5 days. Wound edges were inspected daily and treated with Betadine (10% povidone–iodine) and topical antibiotic.

2.2. Cranial-mounted subcutaneous implant

2.2.1. Connectors

The cranial-mounted subcutaneous implant uses a high density, miniature circular connector system (Amphenol pin, 220-P03-2-100; Amphenol male connector, 222-11n61), as described in Park et al. (2000). Forty multi-stranded stainless steel wires (Cooner Wire, AS632) were cut to lengths appropriate for the 20 pairs of EMG wires to be implanted and the connector was assembled as described in Park et al. (2000).

2.2.2. Surgical protocol

The pre-surgical procedures and anesthesia were the same as described for the arm-mounted subcutaneous implant. In an earlier unrelated surgery, a 50 mm diameter chamber for microelectrode recording was attached to the skull using titanium screws and dental acrylic (Park et al., 2000). One month prior to the EMG implant, a preparatory surgery was performed to hollow out the existing dental acrylic on the head, forming a seat for the connector base. At the same time, two channels were made in the acrylic from the base to the edge of the acrylic just above the occipital ridge of the skull. The preparatory procedure and the EMG implant procedure were separate to decrease the individual surgery length.

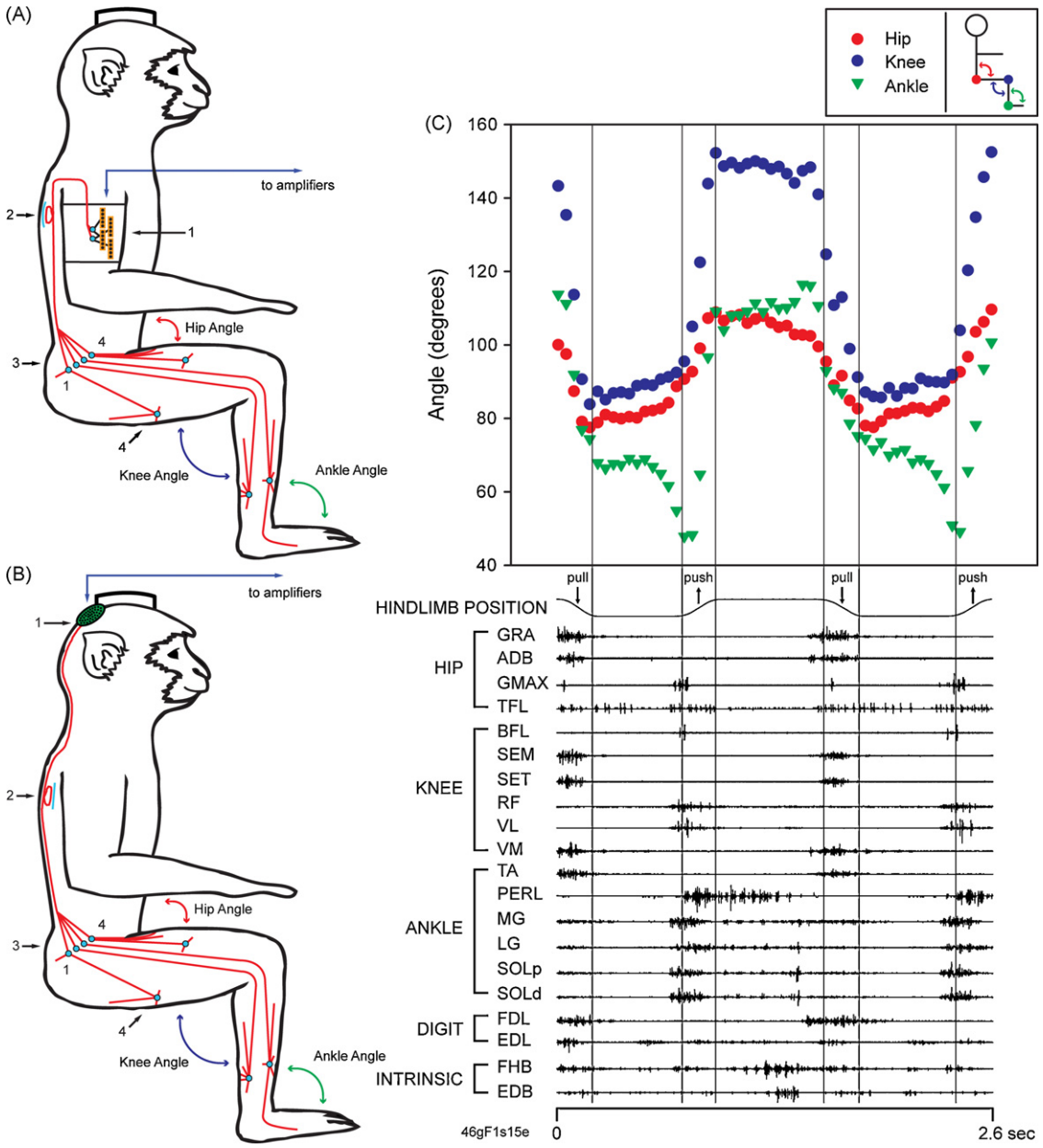

Fig. 3. (A) Arm-mounted subcutaneous implant. Orange rectangles on forelimb: exteriorized connectors that allow connection of EMG wires to amplifiers. Short black lines: exterior wires leading to connectors. Red lines: subcutaneous paths of EMG wires to individual target muscles, wires continuing onto medial side of hindlimb go to target muscles. Light blue circles: sites of small incisions during tunneling. Long light blue line: back pocket where wire loop is seated. Black arrows: 1: exterior arm connectors and entry point of EMG wires, 2: back incision, 3: puncture incisions on the hip, and 4: puncture incision on lateral hindlimb for wires tunneled from hip incision 1. Hip incisions (arrow 3) are numbered 1–4 from most lateral to most medial. (B) Cranial-mounted subcutaneous implant. Red lines, light blue circles and light blue line as described above. Green circle: EMG connector, affixed to skull with dental acrylic, connects to amplifiers. Black arrows: 1: cranial connector and entry point of EMG wires, 2: back incision, 3: puncture incisions on the hip, and 4: puncture incision on lateral hindlimb for wires tunneled from hip incision 1. Hip incisions (arrow 3) are numbered 1–4 from most lateral to most medial. (C) EMG records and joint angle during two cycles of the hindlimb push–pull task. Angle measurement at each joint is described in graph legend and also depicted in (A) and (B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

At the beginning of the EMG implant surgery, the base of the male connector with 40 wires was placed in the seat formed in the dental acrylic. The wires were collected into the channels (proximal muscles in one channel, distal muscles in the second) and directed caudally to the acrylic/skin junction at the occipital ridge (arrow 1, Fig. 3B). Using the custom stainless steel tunneling needles as described above, all wires were passed subcutaneously from the acrylic/skin junction at the occipital ridge to an incision (~4 cm) near the midpoint just below the shoulder blades (arrow 2, Fig. 3B). All wires were exteriorized at this incision and separated into four bundles, two proximal and two distal. Wires were tunneled and implanted as described above in the arm-mounted subcutaneous implant method. When all wires were implanted successfully, each was pulled in both directions at the back incision to take up any extra length. Loops of wire 8–10 cm in length remained at the back incision and were tucked into a subcutaneous pocket before suturing. This was important to ensure sufficient extra wire length to accommodate movements of the leg and head that could not be imposed at the time of surgery. The skull connector and wires were then anchored in place with new dental acrylic. Anchoring of wires at the back incision and all subsequent suturing and sealing of incisions were the same as described in the arm-mounted subcutaneous method. Fig. 3B illustrates the wiring routes. Implanting 20 pairs of EMG leads in

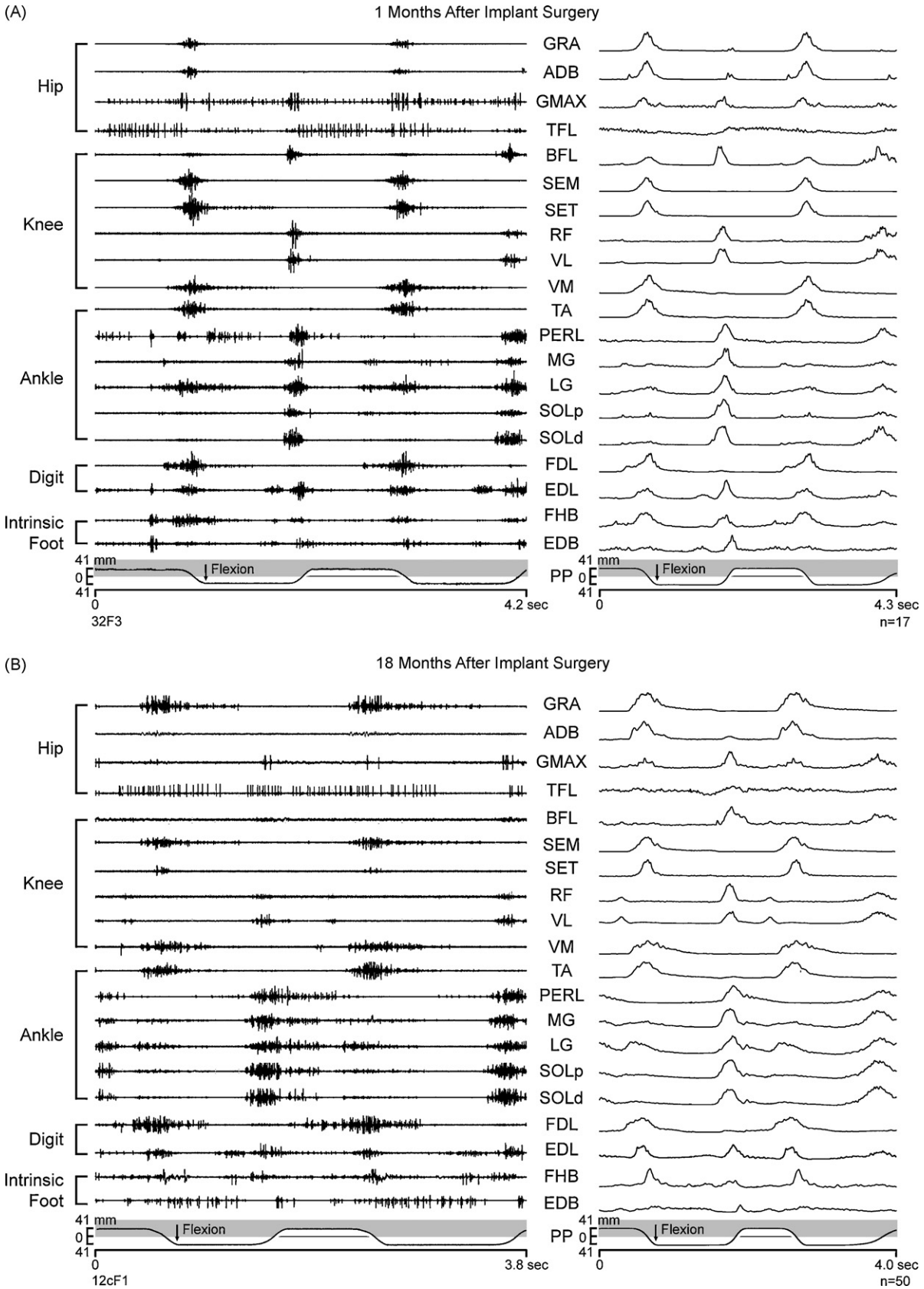

Fig. 4. EMG signals (left) and response averages (right) from 20 pairs of EMG wires implanted in hindlimb muscles at 1 month (A) and 18 months (B) following the arm-mounted subcutaneous implant in Monkey F. The records shown are from two consecutive trials of the push–pull task. Different channels of EMG activity were amplified from 5 to 200 K. Filtering was generally 30 Hz to 3 kHz. All channels were digitized at 4 kHz. Muscle abbreviations are given in Section 2. PP: push–pull task. Shaded area indicates extension position zone. Calibration bar to the left of the task signal indicates linear distance of foot movement during the push–pull task.

Muscle	Joint	Primary action[a]	Secondary action[a]	Stimulus evoked response
GRA	**Hip** Knee[b]	Hip adduction	Knee flexion Medial rotation	Knee flexion Medial rotation
ADB	**Hip**	Hip adduction	Lateral rotation	Hip adduction
GMAX	**Hip** Knee[b]	Hip extension	Hip abduction Knee flexion	Hip extension
TFL	**Hip**	Hip abduction		Hip flexion
BFL	**Knee** Hip[b]	Knee flexion	Hip extension Lateral rotation Hip abduction	Hip extension Knee flexion
SEM	**Knee** Hip[b]	Knee flexion	Hip extension Medial rotation	Knee flexion Medial rotation
SET	**Knee** Hip[b]	Knee flexion	Hip extension Medial rotation	Knee flexion Medial rotation
RF	**Knee** Hip[b]	Knee extension	Hip flexion	Knee extension
VL	**Knee**	Knee extension		Knee extension
VM	**Knee**	Knee extension		Knee extension
TA	**Ankle**	Ankle flexion	Foot inversion	Ankle flexion
PERL	**Ankle**	Foot eversion	Ankle extension	Foot eversion
MG	**Ankle**	Ankle extension		Ankle extension (fast)
LG	**Ankle**	Ankle extension		Ankle extension (fast)
SOLp	**Ankle**	Ankle extension		Ankle extension (slow)
SOLd	**Ankle**	Ankle extension		Ankle extension (slow)
FDL	**Digits** Ankle[b]	Digit flexion	Foot adduction	Digit 2–5 flexion
EDL	**Digits** Ankle[b]	Digit extension	Ankle flexion	Digit 4–5 extension
FHB	**Intrinsic foot**	Hallux flexion		Hallux flexion
EDB	**Intrinsic foot**	Digit 2–5 extension	Digit abduction	Digit 3–4 extension

[a] Primary and secondary actions as listed in Howell and Straus (1971).
[b] Biarticulate, primary joint listed in bold.

hindlimb muscles with this method required approximately 11 h of surgery.

Post-surgical care was the same as described above in the arm-mounted subcutaneous implant method.

3. Results

During surgery, each implanted wire pair was tested for accurate location by sending brief stimulus trains through the wire electrodes and observing evoked movements. These evoked movements were compared to the primary and secondary actions of each muscle as described in Howell and Straus (1971). The target muscle actions and evoked movements during surgery are summarized in Table 1. All muscle responses matched expectations except TFL. However, the evoked movement in TFL was consistent in both monkeys. Stimulus thresholds for evoked movements typically ranged from 0.6 V to 10 V. The cranial-mounted implant was retested with stimulation several times throughout the life of the implant. Thresholds typically remained below 20 V and evoked responses were highly stable.

The push–pull task was designed to engage both proximal and distal hindlimb muscles in reliable and stereotyped patterns of activation. Fig. 3C shows the activation of each implanted muscle with respect to the action of the hindlimb at different phases of the task. The graph of joint angles throughout the push–pull task shows the greatest change in angle at the knee and the least change in angle at the hip. This may explain the broad activation of some hip muscles while the knee muscles were more phasic. Extensor and flexor muscles showed reciprocal patterns of activation, with extensors primarily active during the extension phase of the task and flexors primarily active during the flexion phase of the task. VM, while expected to show EMG activity during the extension phase based upon muscle anatomy, was primarily active during the flexion phase. This may have been due to task design and was consistent in both monkeys and over time. BFL, while primarily a knee flexor, has a secondary action of hip extension and was most active in the extension phase of the task. The digit and intrinsic foot muscles showed broad activation throughout the task.

Cross-talk is more likely to occur between muscles that are adjacent to each other. With the larger size of the hindlimb (compared to the forelimb), we were able to achieve greater separation between electrode pairs. When implanting in adjacent muscles, we made an effort to stagger electrode placement so electrode pairs were not directly adjacent to each other. In the two implants described in this paper, cross-talk was absent in almost all cases. Monkey C showed some cross-talk between LG and SOL, but the magnitude was less than the criterion for rejection.

The arm-mounted subcutaneous implant was used in Monkey F. This implant remained fully viable at 31 months, at which point data collection ended. At regular intervals (approximately every 2 weeks), the monkey was tranquilized to remove the old elastomeric tape, shave the forelimb of hair and affix new tape to the external connectors. Fig. 4 shows samples of EMG activity from all 19 muscles obtained with this method at 1 month (A) and 18 months (B) following implantation. The left column shows a sample of raw EMG from each muscle during two consecutive trials of the push–pull task. The right column shows EMG activity from each muscle averaged over a number of trials. After 18 months, all EMG electrodes were functional. Comparison of the 1- and 18-

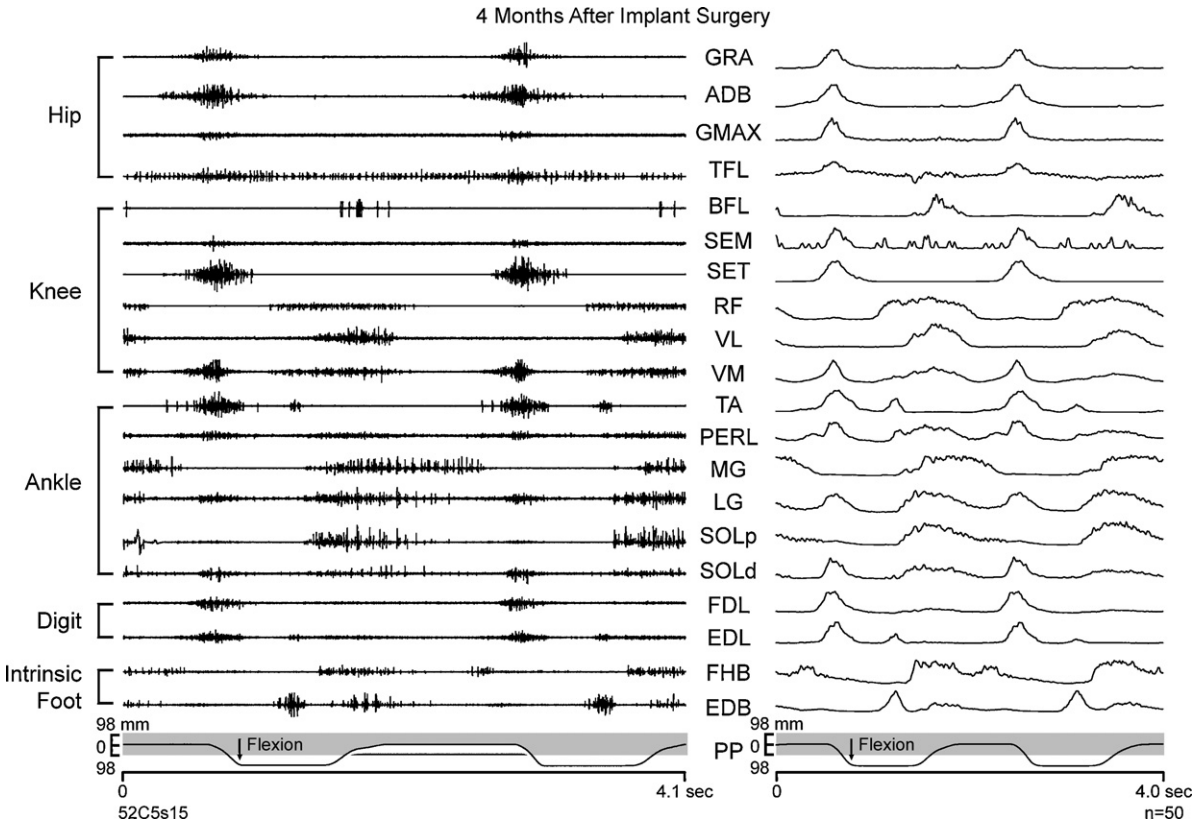

Fig. 5. EMG signals (left) and response averages (right) from 20 pairs of EMG wires implanted in hindlimb muscles at 4 months following the cranial-mounted subcutaneous implant in Monkey C. The records shown are from two consecutive trials of the push–pull task. Different channels of EMG activity were amplified from 5 to 200 K. Filtering was generally 30 Hz to 3 kHz. All channels were digitized at 4 kHz. Muscle abbreviations are given in Section 2. PP: push–pull task. Shaded area indicates extension position zone. Calibration bar to the left of the task signal indicates linear distance of foot movement during the push–pull task.

month records shows a very consistent pattern of activation across all muscles during performance of the push–pull task.

The cranial-mounted subcutaneous implant was used in Monkey C. This implant remained viable for 12 months. Fig. 5 shows samples of EMG activity from all 19 muscles 4 months after implantation. EMG activity patterns were consistent with those obtained with the arm-mounted subcutaneous implant. Four muscles were lost when a small ulceration of the skin developed over a site where several EMG wires were tunneled on the lateral aspect of the distal hindlimb. The ulceration allowed the monkey access to the wires, thus compromising this section of the implant.

Both implant methods were relatively non-traumatic. Each monkey recovered quickly from the surgical procedure and was able to perform the behavioral task a few days after surgery.

4. Discussion

This paper presents two chronic EMG implant methods that are minimally invasive, relatively non-traumatic and capable of recording from 19 muscles of the hindlimb in awake behaving monkeys for periods up to 31 months. Several advantages are common to both methods. The procedure of tunneling the wires subcutaneously adds stability to the implant as the fascia bonds well to the wires' insulation. Tunneling to a point beyond the target muscle and then back proximally to insert wires in the muscle also aids in anchoring the wire in the fascia. The creation of a hook at the tip of the wire, which is then implanted in the muscle belly, increases the stability of the EMG wire in the muscle. The placement of loops of wire in the pocket on the back along with the lengthening of the hindlimb after implantation ensures that the full range of motion was accommodated. Risk of infection and edema is minimized by the very small incision sizes and flushing of the wire pocket on the back with antibiotic (injectable liquid penicillin). Trauma was minimized as well. The monkeys were able to perform behavioral tasks within a few days of the surgery. At necropsy, efforts were made to forcibly remove the implant but there was no give, particularly at the loop on the back, demonstrating how firmly embedded the wires had become. Dissection along the course of the wires showed no evidence of inflammation.

There are advantages and disadvantages to both implant methods. A major benefit of the arm-mounted implant is its modular division and lack of permanence. Parts of the connector can be removed and re-implanted if necessary. The implant is not one unit but rather divided into four individual units. If a problem occurs with a single connector, that connector can be removed and re-implanted rather than the entire EMG implant. The arm-mounted subcutaneous implant is also a shorter surgery than the cranial-mounted subcutaneous implant. The major disadvantage is that the stability of the implant depends upon anchoring the modular connectors to the forelimb with elastomeric tape. With time and hair growth, the adhesiveness of the tape deteriorates. This increases the possibility of the implanted wires becoming dislodged. The tape must be replaced and the forelimb shaved on a regular basis. The jacket, while effective as a protective barrier for the modular connectors, is expensive and requires a significant amount of maintenance due to constant probing and manipulation by the monkey.

The cranial-mounted subcutaneous implant also has advantages and disadvantages. The implant is completely subcutaneous. The head connector is durable and non-removable. The monkey has no access to the implant (barring any unforeseen circumstances such as the skin ulceration described in the methods). Another major advantage is that re-taping the forelimb and the use of a protective

jacket is unnecessary. One disadvantage is the possibility of infection at the acrylic/skin junction on the back of the head where the wires first become subcutaneous. Several strategies were implemented to reduce the possibility of infection at the skin edge near the cortical chamber. (1) Ample time was allowed following the cortical chamber implant surgery for the skin edge to heal. This usually requires at least one and a half months. (2) The bundle of wires leading from the cranial connector was split into two bundles decreasing the size of the skin opening at the edge of the cranial implant. (3) The wires were tunneled deep and close to the skull at the skin–acrylic interface to minimize the chances that any infectious superficial debris was able to enter the subcutaneous wire tunnel. (4) Antibiotics were given until the skin edge healed. (5) Care was taken to avoid disturbing the surface of the skin edge allowing the skin to create its own natural defenses against infection (e.g. scabbing). The length of the implant surgery is another disadvantage. However, by dividing the head connector into two modules, this implant can be accomplished in two much shorter surgeries. This method is also less flexible than the arm-mounted subcutaneous implant method in terms of re-implantation. If there is electrode failure resulting in loss of a viable EMG signal from a muscle or muscles, they cannot be easily recovered using this implant method.

In summary, this paper reports two chronic EMG implant methods, the arm-mounted subcutaneous implant and the cranial-mounted subcutaneous implant. Both methods have individual advantages and disadvantages. However, both methods have been shown to have minimal risk of infection, are relatively non-traumatic and provide stable, long-term EMG recording from large numbers of muscles of the hindlimb in awake behaving monkeys.

Manufacturers and suppliers

A list of manufacturers and suppliers can be found in Park et al. (2000). The following represents any updates or changes.

Stainless steel mesh: Whiting and Davis, 200 John Dietsch Boulevard, Attleboro Falls, MA 02763, United States, Tel.: +1 508 699 4412.

Acknowledgements

This work was supported by NIH Grants NS051825, NS064054 and NIH Center Grant HD025028.

References

Belhaj-Saïf A, Fourment A, Maton B. Adaptation of the precentral cortical command to elbow muscle fatigue. Exp Brain Res 1996;111:405–16.

Bretzner F, Drew T. Contribution of the motor cortex to the structure and the timing of hindlimb locomotion in the cat: a microstimulation study. J Neurophysiol 2005;94:657–72.

Courtine G, Roy RR, Hodgson J, McKay H, Raven J, Zhong H, et al. Kinematic and EMG determinants in quadrupedal locomotion of a non-human primate (Rhesus). J Neurophysiol 2005;93:3127–45.

Drew T. Motor cortical cell discharge during voluntary gait modification. Brain Res 1988;457:181–7.

Fetz EE, Cheney PD. Postspike facilitation of forelimb muscle activity by primate corticomotoneuronal cells. J Neurophysiol 1980;44:751–72.

Hodgson JA, Wichayanuparp S, Recktenwald MR, Roy RR, McCall G, Day MK, et al. Circadian force and EMG activity in hindlimb muscles of rhesus monkeys. J Neurophysiol 2001;86:1430–44.

Hoffer JA, Loeb GE, Marks WB, O'Donovan MJ, Pratt CA, Sugano N. Cat hindlimb motoneurons during locomotion. I. Destination, axonal conduction velocity, and recruitment threshold. J Neurophysiol 1987;57:510–29.

Hoffer JA, Caputi AA, Pose IE, Griffiths RI. Roles of muscle activity and load on the relationship between muscle spindle length and whole muscle length in the freely walking cat. Prog Brain Res 1989;80:75–85, discussion 57–60.

Howell AB, Straus WL. The muscular system. In: Hartman CG, Straus WL, editors. The anatomy of the rhesus monkey. New York: Hafner Publishing Company; 1971.

Loeb GE. Asymmetry of hindlimb muscle activity and cutaneous reflexes after tendon transfers in kittens. J Neurophysiol 1999;82:3392–405.

Miller LE, van Kan PL, Sinkjaer T, Andersen T, Harris GD, Houk JC. Correlation of primate red nucleus discharge with muscle activity during free-form arm movements. J Physiol 1993;469:213–43.

Park MC, Belhaj-Saïf A, Cheney PD. Chronic recording of EMG activity from large numbers of forelimb muscles in awake macaque monkeys. J Neurosci Methods 2000;96:153–60.

Prochazka A, Schofield P, Westerman RA, Ziccone SP. Reflexes in cat ankle muscles after landing from falls. J Physiol 1977;272:705–19.

Prochazka A, Trend P, Hulliger M, Vincent S. Ensemble proprioceptive activity in the cat step cycle: towards a representative look-up chart. Prog Brain Res 1989;80:61–74, discussion 57–60.

Recktenwald MR, Hodgson JA, Roy RR, Riazanski S, McCall GE, Kozlovskaya I, et al. Effects of spaceflight on rhesus quadrupedal locomotion after return to 1G. J Neurophysiol 1999;81:2451–63.

Wolpaw JR, Herchenroder PA. Operant conditioning of H-reflex in freely moving monkeys. J Neurosci Methods 1990;31:145–52.