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# Effects of chronic nerve cuff and intramuscular electrodes on rat triceps surae motor units

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**Abstract**

In order to assess the long-term effects of implanted electrodes on motor unit properties, we studied triceps surae (TS) motor units in rats implanted for 3–10 months with a tibial nerve cuff electrode for H-reflex elicitation and intramuscular electrodes for recording TS electromyographic activity. Motor units with sag from implanted rats displayed greater tetanic force than those from unimplanted rats. Motor units without sag had shorter twitch contraction times. This disrupted the relationship between sag and contraction time that was always present in unimplanted rats. These differences were consistent with a small degree of muscle denervation and subsequent reinnervation. Further analyses ascribed this effect to the nerve cuff rather than to the intramuscular electrodes. Comparable changes in motor unit properties may occur in humans with implanted nerve cuffs. © 2001 Published by Elsevier Science Ireland Ltd.

*Keywords*: Motor units; Implanted electrodes; H-reflex; Reinnervation; Nerve cuff; Chronic recording; Contraction time; Sag

Rats and monkeys can gradually increase or decrease the triceps surae (TS) H-reflex (the electrical analog of the spinal stretch reflex produced by homonymous and synergist group I afferent excitation of the motoneuron) over weeks without change in background electromyographic activity (EMG) or M-response (the EMG produced by direct motoneuron axon activation) when they are exposed to an operant conditioning protocol in which reward depends on H-reflex size [19]. This phenomenon is associated with physiological and anatomical plasticity at several sites in the spinal cord [19]. Conditioning-induced alterations in motoneuron firing behavior could produce activity-dependent changes in motor unit properties [8,9]. This could affect the number and/or firing rates of the motor units that comprise the fixed level of background EMG activity that precedes H-reflex elicitation and/or might affect the recruitment of motor units into the H-reflex [10]. However, axonal damage by the chronically implanted nerve cuff and EMG electrodes [11,12,17] might confound evaluation of this possibility [3]. Implantation and/or continued exposure to implanted electrodes could denervate muscle fibers and lead to their subsequent reinnervation by other motor axons [2,13]. Motor unit properties can change after reinnervation due to differences in activity pattern or trophic influences [8].

To assess this possibility, the present study compares the motor unit properties of chronically implanted (IMP) rats with those of unimplanted (CON) rats. All procedures conformed to NIH guidelines on animal care and were reviewed and approved by the Institutional Animal Care and Use Committee of the Wadsworth Center. Using previously described methods [5–7,19], seven male Sprague–Dawley rats (308–475 g) were anesthetized (80 mg/kg ketamine + 10 mg/kg xylazine) and implanted in the right leg with three pairs of Teflon-insulated multistranded stainless steel wires (insulated diameter, 235 μm; bare wire diameter, 75 μm). One pair, secured to the inner circumference of a 4-mm length of silicone rubber tubing [5–6], formed a bipolar stimulation cuff that was placed on the posterior tibial nerve just proximal to the TS muscles (i.e. medial gastrocnemius muscle (MG), lateral gastrocnemius muscle (LG), and soleus muscle (SOL)). The other two pairs of electrodes were inserted in SOL (five rats) or one pair in SOL and the other pair in MG and LG (two rats) for recording EMG. All wires passed subcutaneously to a skull-mounted plug and then through a flexible cable and a commutator to stimulation and recording equipment. SOL EMG was monitored continuously by a computer, which also delivered a nerve cuff stimulus whenever background EMG remained

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within a defined range for a randomly varying 2.3–2.7 s period and maintained the nerve cuff stimulus at M-response threshold (average of 3000–5500 stimuli/day in five of seven rats; 919 and 250 stimuli/day in the two other rats).

Rats were healthy and active during the several months of data collection. Mean implant duration ± SD was 178 ± 93 days (range = 91–314 days). H-reflex size remained stable throughout the recording period. Mean H-reflex size ± SEM for the last 10 days of data collection averaged 101 ± 4% of its value for the first 10 days. M-response size and background EMG remained stable throughout.

Rats were then anesthetized (60 mg/kg pentobarbital initially, supplemented with 10 mg/kg as needed) and prepared for motor unit study as described previously [3,4]. TS muscle force was recorded by a force transducer connected to the common tendon. EMG was recorded by pairs of fine-wire electrodes in each of the three TS muscles. Electroneurographic activity (ENG) was recorded from a sub-epineurial electrode on the tibial nerve at the level of the knee (i.e. just proximal to the nerve cuff electrode in IMP rats). L5 or L6 ventral root axons were impaled with glass microelectrodes and were identified by all-or-none EMG and twitch responses to single brief current injections over at least a 4-fold range of intensity.

Twitch force, EMG, and axon potential were recorded during 0.2-Hz stimulation and averaged for determination of maximum twitch force, twitch contraction time, and EMG latency (i.e. time from axonal action potential initiation until EMG onset using the electrode pair having the largest response during the twitch). Force was recorded during a 600-ms, 200-Hz train of stimuli repeated 5–10 times at 10-s intervals for determination of maximum tetanic force. To detect sag (i.e. a transient decrease in force during unfused tetanic contraction), force was recorded during at least four trains of 25 stimuli with inter-stimulus intervals of 1–4 times the twitch contraction time. Motor units with sag were classified as fast twitch (F); those without were classified as slow twitch (S). Endurance was assessed by recording force during 70-Hz trains of 14 stimuli delivered at 1-s intervals for 2 min and calculating a fatigue index (i.e. the ratio of the maximum force during the 120th tetanus to that during the largest of the first 10 tetani). The fatigue index classified F motor units as: fatigue-resistant (FR; fatigue index$\geq$ 0.75), having intermediate fatigability (Fint; 0.25 $<$ fatigue index $<$ 0.75), or fatigue-sensitive (FF; fatigue index $\leq$ 0.25). Axonal conduction velocity was calculated as the ratio of the conduction distance to the conduction time (i.e. onset of current-evoked action potential to onset of the largest negative ENG component determined from the average of 400–4000 intra-axonal and tibial nerve responses).

We compared 197 motor units from the seven IMP rats to 165 motor units from seven CON rats (79 CON motor units from Carp et al. [4]) of comparable body weight (mean ± SD = 620 ± 64 g and 577 ± 45 g, respectively). Data from each rat consisted of 11–37 motor units (median = 26). Motor units for which the EMG signal was largest in either MG or LG were treated as a single group of motor units (MG/LG); motor units for which the EMG signal was largest in SOL were combined with the MG/LG units to comprise a single group of TS motor units. A separate SOL group was not used because of the risk of crosstalk from the MG and LG fibers that were unavoidably close to the SOL EMG wires.

Fig. 1 shows the relationship between contraction time and sag during unfused tetani for CON (upper bars) and IMP (lower bars) rats. In CON rats, sag and contraction time identified the same motor units as F or S type. Motor units with sag (clear bars) always had contraction times $<$ 20 ms, and those without sag (hatched bars) always had contraction times $>$ 20 ms. In contrast, some motor units of IMP rats showed a mismatch between sag and contraction time. Ten motor units from six of seven rats had contraction times $<$ 20 ms but no sag, and one motor unit with sag had a contraction time $>$ 20 ms. The frequency of mismatch was significantly higher in IMP than in CON rats (11 of 151 and 0 of 127 motor units in which both sag and contraction time were evaluated for IMP and CON rats, respectively; $P = 0.001$, $\chi^2$), nor were mismatches observed in a larger sample of motor units from unimplanted rats [4]. Similar mismatches between sag and contraction time were observed in nerve-damaged, but not in intact cat tibialis anterior muscles [15]. These mismatches were attributed to the incomplete conversion of muscle properties after re-innervation by a foreign motoneuron. This suggests that some muscle denervation occurred in IMP rats.

Only FR and S motor units displayed the sag-contraction time mismatch. Tetanic force, conduction velocity, and fati-

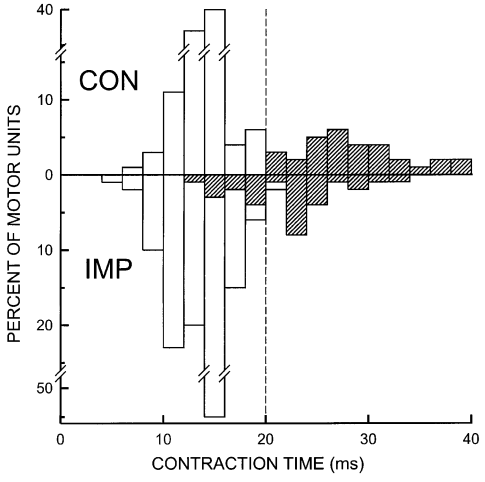


Fig. 1. Distributions of contraction times in motor units with sag (clear bars) or without sag (hatched bars) in CON rats (upward bars) and IMP rats (downward bars) as percent of the total number of motor units in each group. In CON rats, all motor units without sag had contraction times $>$ 20 ms and all motor units with sag had contraction times $<$ 20 ms. In IMP rats, some motor units showed a mismatch between sag and contraction time: ten motor units without sag had contraction times $<$ 20 ms, and one motor unit with sag had a contraction time $>$ 20 ms.

Table 1
Properties of F and S motor units in CON and IMP rats[a]

| Type | Property | CON | IMP |
|---|---|---|---|
| F | Tetanic force (mN) | 171 ± 26 | 242 ± 23* |
| | Contraction time (ms) | 13.4 ± 0.5 | 13.2 ± 0.5 |
| | Conduction velocity (m/s) | 71.5 ± 1.3 | 73.6 ± 1.6 |
| | EMG latency (ms) | 2.35 ± 0.06 | 2.59 ± 0.06* |
| | Fatigue index | 0.58 ± 0.04 | 0.55 ± 0.04 |
| S | Tetanic force (mN) | 47 ± 6 | 56 ± 5 |
| | Contraction time (ms) | 29.2 ± 1.5 | 21.8 ± 1.4† |
| | Conduction velocity (m/s) | 62.7 ± 1.7 | 66.0 ± 1.8 |
| | EMG latency (ms) | 3.03 ± 0.19 | 3.14 ± 0.20 |
| | Fatigue index | 1.00 ± 0.01 | 0.99 ± 0.01 |

[a] Values are means ± SEM of each rat's average values for F or S motor units. *, different from F motor units of CON rats at $P < 0.05$ by nested analysis of variance (rats nested within treatment groups and measurements from individual motor units nested within rats); †, different from S motor units of CON rats at $P < 0.001$ by nested analysis of variance. Numbers of F and S motor units were 94–101 and 33–34, respectively, from seven CON rats and 121–124 and 31–33, respectively, from seven IMP rats for all properties, except for conduction velocity (73 and 19 F and S motor units, respectively, from CON rats and 109 and 23 F and S motor units, respectively, from IMP rats).

gue index of the ten mismatched motor units with short contraction times but no sag (mean ± SEM = 60 ± 7 mN, 67.8 ± 1.5 m/s, and 1.00 ± 0.01, respectively) were not significantly different from those of S motor units (62 ± 9 mN, 63.3 ± 2.8 m/s, and 0.99 ± 0.01), but were significantly different from those of FR motor units (126 ± 25 mN, 74.3 ± 1.9 m/s, 0.89 ± 0.01; $P < 0.05$ vs. mismatched group, ANOVA). Because the properties of the mismatched motor units (with the exception of contraction time) are consistent with the S rather than the FR profile, subsequent analyses treat the 10 motor units without sag but with short contraction times as S. (The one motor unit with sag and a contraction time > 20 ms was not included in further analyses.)

To evaluate the effect of the chronic EMG electrodes, properties of the 34 MG/LG motor units from the two rats with both SOL and MG/LG EMG wires were compared with those of the 101 MG/LG motor units from the five rats with only SOL EMG wires. There were no significant differences between intact and implanted MG/LG muscles in maximum tetanic force or fatigue index for all motor units or for F motor units alone ($P > 0.5$ for all, ANOVA; there were too few S motor units for meaningful analysis), or in motor unit type or sag-contraction time mismatch distributions ($P > 0.3$ for both, $\chi^2$). The lack of difference in force measurements between implanted and intact MG/LG muscles and the lack of a greater degree of mismatched motor units in the implanted muscles suggest that the implantation and continued presence of the EMG recording wires did not affect motor unit properties.

To assess the effect of implantation of the chronic tibial nerve cuff on motor unit properties, data from all TS muscles were pooled and compared with those from CON rats. Assuming that the lack of effect of EMG wire implantation on MG/LG motor unit properties also held true for implantation of electrodes in SOL, any implantation-related changes would be attributable to the nerve cuff. Table 1 compares properties of F and S motor units from CON and IMP rats. The tetanic force of F motor units was significantly larger in IMP rats than in CON rats, due primarily to the greater proportion of the largest motor units in IMP rats than in CON rats. Fig. 2 shows that 17% and 6% of motor units had tetanic force > 400 mN, respectively ($P < 0.002$, $\chi^2$ test), and 7% of the motor units from IMP rats had tetanic forces larger than any motor units from CON rats. This is consistent with an expansion of motor unit size after denervation [2,13].

Contraction times of S motor units were significantly shorter in IMP rats than in CON rats. Although the short contraction times of the ten motor units with sag-contraction time mismatches contributed to this difference, their exclusion did not alter the difference in contraction time between IMP and CON rats (mean contraction time ± SEM = 24.5 ± 0.8 for IMP S motor units without mismatched units; $P = 0.008$, IMP vs. CON rats, ANOVA). This suggests that the effect of the nerve cuff was not restricted to the few motor units that displayed a mismatch between contraction time and sag.

EMG latency, but not axonal conduction velocity, of F motor units was significantly longer in IMP rats than in CON rats. Because the conduction path measured by EMG latency includes that measured by conduction velocity, the difference in EMG latency probably reflects slowing in axonal conduction distal to the ENG recording electrode. This is consistent with an effect of the nerve cuff (which was located distal to the ENG electrode), despite the fact that its inside diameter was at least twice the nerve diameter [11,17]. Indeed, damage may accrue throughout the duration of implantation. Among IMP rats,

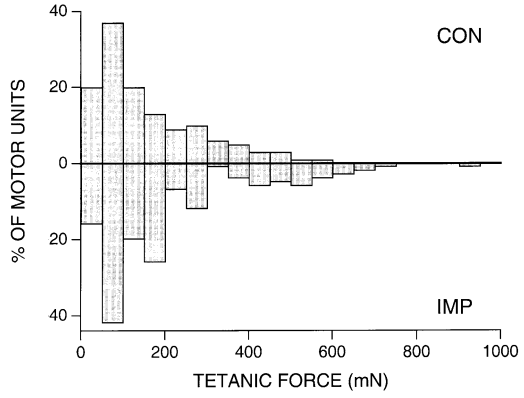


Fig. 2. Distributions of tetanic force in motor units of CON rats (upward bars) and IMP rats (downward bars) as a percent of the total number of motor units in each group. IMP rats have a greater proportion of motor units with large tetanic forces than CON rats (see text for statistical analysis).

EMG latency varied directly with implant duration, but not with body weight ($P = 0.04$ and $P = 0.47$ for slopes of regression of average EMG latency in each rat on implant duration and body weight, respectively). This slowing could reflect a focal effect due to compression by the cuff or slower conduction by new axon sprouts. The preferential increase in the largest motor unit tetanic forces and the decrease in contraction time of S (but not F) motor units are consistent with preferential damage to motor units with larger axons and a shift in S motor unit contractile properties triggered by the reinnervation of denervated muscle fibers. Nevertheless, any nerve damage that does occur is clearly limited, given the modest changes that occur in motor unit properties and the preservation of reflex function in IMP rats and in the much larger population of conditioned animals with implanted nerve cuffs [5–7].

The head-mounted tether system with its lightweight cable and low-torque commutator did not appear to interfere with motor function. However, we cannot exclude the possibility that the tethered (i.e. IMP) rats needed more muscle activation than CON rats to produce the same movements, causing activity-dependent changes in contractile properties [8].

Repeated application of the low-intensity stimulus that elicits the H-reflex could in theory contribute to activity-dependent changes in motor unit contractile properties. The total number of stimuli applied to the tibial nerve in IMP rats varied widely (range = 11,241–472,791 stimuli for 24–225 days). However, the lack of any significant linear relationships between stimulus number and any motor unit properties studied here and the low frequency of stimulus application ($< 0.36$ Hz) indicated that the H-reflex stimulus itself was not responsible for the differences in motor unit properties between CON and IMP rats.

Our data support the hypothesis that modest denervation due to the nerve cuff and subsequent reinnervation by adjacent intact motor units was responsible for the differences in motor unit properties between IMP and CON rats. In addition to their importance for our studies of H-reflex conditioning and other animal studies using chronically implanted electrodes, these results are relevant to clinical neuroprosthetic applications of this methodology. Proper function of these devices depends in part on stable relationships between nerve stimulation and muscle output. Thus, alterations in motor unit force or EMG due to implanted electrodes or cuffs may need to be taken into account in the design of neuroprosthetic control strategies [1,14,16,18].

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[1] Bhadra, N. and Peckham, P.H., Peripheral nerve stimulation for restoration of motor function, J. Clin. Neurophysiol., 14 (1997) 378–393.
[2] Brown, M.C. and Ironton, R., Sprouting and regression of neuromuscular synapses in partially denervated mammalian muscles, J. Physiol., 278 (1978) 325–348.
[3] Carp, J.S., Chen, X.Y., Sheikh, H. and Wolpaw, J.R., Motor unit properties after operant conditioning of rat H-reflex, Exp. Brain Res, (2001) in press.
[4] Carp, J.S., Herchenroder, P.A., Chen, X.Y. and Wolpaw, J.R., Sag during unfused tetanic contractions in rat triceps surae motor units, J. Neurophysiol., 81 (1999) 2647–2661.
[5] Chen, X.Y. and Wolpaw, J.R., Dorsal column but not lateral column transection prevents down-conditioning of H reflex in rats, J. Neurophysiol., 78 (1997) 1730–1734.
[6] Chen, X.Y. and Wolpaw, J.R., Operant conditioning of H-reflex in freely moving rats, J. Neurophysiol., 73 (1995) 411–415.
[7] Feng-Chen, K.-C. and Wolpaw, J.R., Operant conditioning of H-reflex changes synaptic terminals on primate motoneurons, Proc. Natl. Acad. Sci. USA, 93 (1996) 9206–9211.
[8] Gordon, T. and Pattullo, M.C., Plasticity of muscle fiber and motor unit types, Exer. Sport Sci. Rev., 21 (1993) 331–362.
[9] Gordon, T., Tyreman, N., Rafuse, V.F. and Munson, J.B., Fast-to-slow conversion following chronic low-frequency activation of medial gastrocnemius muscle in cats. I. Muscle and motor unit properties, J. Neurophysiol., 77 (1997) 2585–2604.
[10] Jones, K.E. and Bawa, P., A comparison of human motoneuron data to simulated data using cat motoneuron models, J. Physiol. (Paris), 93 (1999) 43–59.
[11] Krarup, C. and Loeb, G.E., Conduction studies in peripheral cat nerve using implanted electrodes: I. Methods and findings in controls, Muscle Nerve, 11 (1988) 922–932.
[12] Larsen, J.O., Thomsen, M., Haugland, M. and Sinkjaer, T., Degeneration and regeneration in rabbit peripheral nerve with long-term nerve cuff electrode implant: a stereological study of myelinated and unmyelinated axons, Acta Neuropathol., 96 (1998) 365–378.
[13] Luff, A.R., Hatcher, D.D. and Torkko, K., Enlarged motor units resulting from partial denervation of cat hindlimb muscles, J. Neurophysiol., 59 (1988) 1377–1394.
[14] Popovic, D.B., Stein, R.B., Jovanovic, K.L., Dai, R., Kostov, A. and Armstrong, W.W., Sensory nerve recording for closed-loop control to restore motor functions, IEEE Trans. Biomed. Eng., 40 (1993) 1024–1031.
[15] Rafuse, V.F. and Gordon, T., Incomplete rematching of nerve and muscle properties in motor units after extensive nerve injuries in cat hindlimb muscle, J. Physiol., 509 (1998) 909–926.
[16] Stein, R.B., Belanger, M., Wheeler, G., Wieler, M., Popovic, D.B., Prochazka, A. and Davis, L.A., Electrical systems for improving locomotion after incomplete spinal cord injury: an assessment, Arch. Phys. Med. Rehab., 74 (1993) 954–959.
[17] Stein, R.B., Nichols, T.R., Jhamandas, J., Davis, L. and Charles, D., Stable long-term recordings from cat peripheral nerves, Brain Res., 128 (1977) 28–38.
[18] Strange, K.D. and Hoffer, J.A., Restoration of use of paralyzed limb muscles using sensory nerve signals for state control of FES-assisted walking, IEEE Trans. Rehab. Eng., 7 (1999) 289–300.
[19] Wolpaw, J.R., The complex structure of a simple memory, Trends Neurosci., 20 (1997) 588–594.