Asymmetry of Hindlimb Muscle Activity and Cutaneous Reflexes After Tendon Transfers in Kittens

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Loeb, G. E. Asymmetry of hindlimb muscle activity and cutaneous reflexes after tendon transfers in kittens. J. Neurophysiol. 82: 3392–3405, 1999. The mechanical actions of various ankle muscles were changed by surgically crossing or transferring the tendons in kittens. After the kittens grew to adults, both hindlimbs were implanted with multiple electromyogram (EMG) recording and cutaneous nerve stimulation electrodes to compare the activity of altered and normal muscles. The tendon transfers showed a remarkable tendency to regrow toward normal or only slightly altered mechanical action. In these animals and in the sham-operation controls, the patterns of muscle activity and reflexes were symmetrical in corresponding muscles of the two legs, although they could differ substantially between animals, particularly for the cutaneous reflexes. Eleven animals had at least some persistent alterations in muscle action. Their cutaneous reflex patterns tended to be asymmetric, in some cases quite markedly. EMG activity during unperturbed locomotion and paw-shaking was more symmetrical, but there were some changes in altered muscles and their synergists. The central pattern generators for locomotion and paw-shaking and particularly for cutaneous reflexes during locomotion appear to be at least partially malleable rather than entirely hardwired. This may provide a tool for studying their development and spinal plasticity in general.

INTRODUCTION

Locomotor patterns of muscle activity in quadrupeds are produced by a central pattern generator (CPG) in the spinal cord and are modified by ongoing sensory feedback. For the major limb muscles, the patterns are reproducible across animals and resistant to change even when the mechanical actions of muscles are changed by surgically crossing the tendons (Forsberg and Svartengren 1983; Sperry 1945).

The locomotor CPG also modulates the gain of various proprioceptive and cutaneous reflexes that can be elicited by natural or electrical stimulation at various points in the step cycle (Andersson et al. 1978; LaBella et al. 1992). The mechanisms for this modulation include changes in the bias polarization of pools of motoneurons innervating particular muscles, excitation and inhibition of interneurons in oligosynaptic pathways from afferents to motoneurons, and presynaptic inhibition of afferent projections to interneurons and motoneurons. These reflexes have been interpreted as playing a useful role in maintaining stability and avoiding injury if the limb encounters a mechanical obstacle or noxious stimulus during its movement cycle. Because of the different mechanical constraints on load-bearing and motion in different phases of locomotion, it seems appropriate for the magnitude and even the nature of the reflex responses to change accordingly. For large, proximal limb muscles, the responses to low-threshold cutaneous stimulation can be divided simply into those that tend to unload the limb during stance phase (extensor inhibition) and those that tend to pull it around or away from an obstacle during swing phase (flexor excitation) (Abraham et al. 1985; Duyens and Loeb 1980; Pratt et al. 1991). Smaller and more distal limb muscles, particularly those operating the ankle and digits, are not so easily divided into flexors and extendors (Young et al. 1993), nor is it obvious whether or how they participate in these unloading and flexion synergies. In fact, their responses to a given stimulus seem to be quite different from animal to animal, suggesting that at least some of these reflex patterns may be learned by the CPG rather than genetically hardwired (Loeb 1993).

The general hypothesis is that the CPG includes functional elements that are capable of learning and adaptation, which gives rise to interanimal variability, as reported in adults. It is not possible to control completely the environment in which an animal learns to locomote, but it seems reasonable to assume that the conditions affect the two hindlimbs symmetrically. The purpose of these experiments was to demonstrate the degree to which natural muscle activity patterns and cutaneous reflexes during locomotion were symmetrical between hindlimbs of a given animal and the degree to which that symmetry could be broken by introducing mechanical asymmetry in the skeletal actions of distal hindlimb muscles. The results confirmed that animals normally recruit their muscles in a symmetrical manner and demonstrated a range of asymmetries that could be induced by surgically altering the mechanical actions of individual muscles at an early age. A preliminary report of these results has appeared previously (Loeb 1998).

METHODS

Twenty newborn kittens of mixed sex were subjected to various surgical interventions under general anesthesia and aseptic conditions. They were allowed to develop normally to adulthood in an open cat colony where they interacted freely with their mothers, siblings, and other adult and juvenile animals in an enriched environment with various toys and surfaces to encourage normal motor development. As young adults, they were given regular training to accustom them to walking steadily in a motor-driven treadmill with Plexiglas enclosure to facilitate videotaping of their movements. After reaching maturity (7–31 mo, mean 15 mo), each animal was implanted with a chronic electrophysiology system consisting of symmetrical arrays of various recording and stimulation electrodes in each hindlimb plus a 40-pin back-pack connector (Hoffer et al. 1987). The various muscles studied, their anatomic locations and mechanical actions in the parasagittal
plane are summarized in Fig. 1A, along with abbreviations used throughout. It is important to remember that many of these muscles have substantial or even predominant actions in the planes of inversion-eversion and internal-external rotation, as can be inferred from a more anatomically detailed view of their tendons (Fig. 1B, adapted from Young et al. 1993, who measured multiaxis moment arms for most of the muscles studied).

Most of the recordings reported here were made in a 2-wk period.
after full recovery from anesthesia and surgery and becoming accustomed to walking with a percutaneous connector and ribbon cable for recording. Shortly thereafter, the animal was killed with an anesthetic overdose and both hindlimbs were explored to determine the mechanical actions and masses of the muscles and their synergists under study and to confirm the surgical placement of the stimulation and recording electrodes (see RESULTS, Fig. 11, for example).

Neonatal surgeries

Microsurgical techniques were used in kittens (8–19 days after birth; n = 18) to change the mechanical action of two muscles by cutting and reattaching the switched tendons (cross) or by cutting one tendon and attaching the muscle to the side of another muscle’s intact tendon (transfer). Nonresorbable sutures with low reactivity (Ethibond, Ethicon) were used throughout and were usually noted at the adult surgery as an aid to identifying the locus of the original surgery and the nature of any reorganization. Any active intervention was always to the right leg; the left leg was subjected to a sham operation, including similar skin incision and sutures applied to the corresponding intact tendons. Table 1 summarizes the various modifications attempted, which included 12 tendon crosses, six transfers, and two control animals with sham operations bilaterally.

Chronic recording techniques

Because the main outcome measure involved the symmetry of electromyographic (EMG) activity and reflexes, considerable pains were taken to assure that the devices and their implantation did not induce any asymmetries into either the behavior or the recorded signals. All EMG recordings were obtained from epimysial patch electrodes with 3-mm interelectrode distances (Loeb and Gans 1986), sutured to the fascial surfaces of at least six matched muscles in each hindlimb (some of the electrodes and leads can be seen in post mortem Fig. 11). The dielectric support material tends to block cross-talk between adjacent muscles and maintains the electrical compartment. The altered right leg was always implanted first so that any changes in the electrode placement could be mirrored in the control left leg. The impedance of all EMG electrode contacts was measured at surgery and before each recording session to test for any deterioration; values were consistently in the range of 1–3 kΩ (test signal 1 µA at 1 kHz).

All animals were implanted bilaterally with bipolar nerve cuff electrodes (4 mm spacing) for stimulating the superficial peroneal and sural nerves and tripolar nerve cuff electrodes (6-mm spacing) for recording evoked potentials from the sciatic nerve. Some of these animals provided data for a parallel study of fabrication methods for nerve cuff electrodes (Loeb and Peck 1996). The integrity of the nerves and positioning of these electrodes was verified after surgery and at each recording session using impedance monitoring and electrophysiological responses. Nerve recordings were made using a transformer-coupled preamplifier (model ADT-1, Micro Probe; 100–10,000-Hz bandwidth). Stimulus-triggered averaging of the sciatic nerve recordings was used to determine the threshold for producing evoked potentials and the latency and amplitude of various waves in the compound action potential at various stimulus levels, expressed here as multiples of the group I threshold (e.g., 2 × 7). Thresholds were generally in the range of 20–40 µA for electrically isolated biphasic pulses (cathode-first at proximal contact, 100 µs/phase).

Multichannel EMG activity (50–5,000 Hz) was recorded on 14-track FM tape along with sciatic nerve activity (100–10,000 Hz), a synchronization and calibration signal for any stimulus current pulses.

Table 1. Surgical modifications attempted and outcomes

<table>
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<tr>
<th>Kitten</th>
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<th>Outcome</th>
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<tbody>
<tr>
<td>TT01</td>
<td>EDL × LG-SOL</td>
<td>Regrew nl</td>
</tr>
<tr>
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<td>PL × SOL</td>
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</tr>
<tr>
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<td>TA × PLA</td>
<td>Regrew nl</td>
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<td>TT04</td>
<td>TA × PLA</td>
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</tr>
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<td>FHL nl, POP → FDL → TP</td>
</tr>
<tr>
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<td>PL × PB</td>
<td>Regrew nl</td>
</tr>
<tr>
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<td>EDL × SOL</td>
<td>EDL small, SOL → EDL</td>
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<td>TT08</td>
<td>TA × MG</td>
<td>Regrew nl</td>
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<tr>
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<td>TP small, FHL large, POP → FDL → TP</td>
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<td>FDL × FHL</td>
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</tr>
<tr>
<td>TT11</td>
<td>PL × PB</td>
<td>PL absent, PB small</td>
</tr>
<tr>
<td>TT12</td>
<td>Bilateral sham</td>
<td>NI</td>
</tr>
<tr>
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<td>TT14</td>
<td>PL × PT</td>
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<td>FDL → TP</td>
<td>Regrew nl</td>
</tr>
<tr>
<td>TT16</td>
<td>FDL → PL</td>
<td>EDL&lt;sub&gt;deep&lt;/sub&gt; nl, EDL&lt;sub&gt;super&lt;/sub&gt; → PL</td>
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<td>FDL → TP</td>
<td>FDL → TP</td>
</tr>
<tr>
<td>TT18</td>
<td>EDL → PL</td>
<td>EDL&lt;sub&gt;super&lt;/sub&gt; nl, EDL&lt;sub&gt;deep&lt;/sub&gt; → PL</td>
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<td>EDL → PL</td>
<td>EDL → PL</td>
</tr>
<tr>
<td>TT20</td>
<td>FDL → TP</td>
<td>FDL → TP</td>
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</tbody>
</table>

See Fig. 1 for definition of abbreviations. Surgery was performed on the right leg, with the left leg serving as the sham-operation control. * →, attached; nl, normal.

Chronic recording techniques

Because the main outcome measure involved the symmetry of electromyographic (EMG) activity and reflexes, considerable pains were taken to assure that the devices and their implantation did not induce any asymmetries into either the behavior or the recorded signals. All EMG recordings were obtained from epimysial patch electrodes with 3-mm interelectrode distances (Loeb and Gans 1986), sutured to the fascial surfaces of at least six matched muscles in each hindlimb (some of the electrodes and leads can be seen in post mortem Fig. 11). The dielectric support material tends to block cross-talk between adjacent muscles and maintains the spacing and alignment of the contacts in relation to the muscle fibers, which tends to standardize the recorded amplitude of the EMG (Loeb 1993; Loeb and Gans 1986). The details of the electrode designs, the surgical approaches, and fixation sites were customized for each animal based on the nature of the original surgery, prior experience with the relevant muscles, and cadaver dissections when necessary. A selection of extra electrodes was kept available at surgery to deal with unusual anatomic variations such as muscles with apparently split mechanical actions, which were then fitted with separate recording electrodes for each functional compartment. The altered right leg was always implanted first so that any changes in the electrode placement could be mirrored in the control left leg. The impedance of all EMG electrode contacts was measured at surgery and before each recording session to test for any deterioration; values were consistently in the range of 1–3 kΩ (test signal 1 µA at 1 kHz).

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Multichannel EMG activity (50–5,000 Hz) was recorded on 14-track FM tape along with sciatic nerve activity (100–10,000 Hz), a synchronization and calibration signal for any stimulus current pulses.
and Society of Motion Picture and Television Engineers time code for synchronization of simultaneously recorded audio and video (right lateral view). Natural activities included treadmill locomotion at a range of speeds and various other behavior such as jumping to and from a 1-m-high ledge and ear-scratching (induced by introducing a drop of water into the ipsilateral ear). Paw-shaking was induced by applying a piece of tape to the toes, which usually elicited one burst of two to five cycles of shaking during each of the subsequent one to three swing cycles of walking. Short-latency cutaneous reflexes were induced by single pulses of electrical stimulation while the cat walked steadily on the treadmill (approx. 0.5–0.8 m/s, cadence 0.65–0.85 s/step). Sequences of 2–3 min of walking were obtained while stimulating every 1.3 s, which tended to scatter the stimuli, with random phases in approximately every other step cycle. Averaged evoked potentials were used to balance the stimulation intensity between the two sides at a standard level of 2 × \(T\) for each nerve (see RESULTS, Fig. 4, A and B for an example). Additional sequences were usually obtained at 1.5 × \(T\) and 3 × \(T\).

**Data analysis**

EMG and sciatic nerve recordings were preprocessed by full-wave rectification and bin integration (1.67- or 3.3-ms bins synchronized to the recorded SMPTE time code for reproducibility) before digitizing at the bin rate by a 16-channel MacAdios A/D converter system in a Macintosh IIFX computer. This form of signal reduction effectively generates the area-under-the-curve of the EMG signal with a one-bin time delay and zero hysteresis between bins. Calibration bars on all traces herein provide signal amplitude referred to input and normalized for a bin integration time of 1 ms; they correspond to unprocessed EMG waveforms with peak-to-peak (p-p) amplitudes approximately 10 times greater (e.g., 100 \(\mu V\) bin corresponds to 1 mV p-p EMG). Custom software was used to key the digitizing process to synchronize pulses denoting stimuli and to SMPTE time codes denoting particular activities as identified by field-by-field playback of the corresponding videotape.

The digitized files were reviewed in a simple chart recorder mode (e.g., RESULTS, Figs. 3A and 6A) and analyzed according to...
step cycles and peristimulus traces by custom Macintosh software. Segments of continuous walking were broken up into step cycles by identifying a reliable feature in an ipsilateral EMG trace, usually the onset of the extensor burst in SO. These step cycles were then arranged according to duration. Cycles were rejected if they were longer or shorter than the normal walking range for the animal and if the previous or following step differed by more than 15% in duration, indicating acceleration or deceleration. Manual rejection was also available for step cycles for which EMG profiles appeared noisy, although this was infrequently applied. Three additional features were then identified within each of the step cycles, based on readily discriminable features of other EMG channels. For a sample step cycle, the EMG features were related to the standard inflections of the Phillipson (1905) step cycle (footfall, midstance, footlift, and midswing) by identifying them kinematically on successive still-field video images. The position of the inflection was calculated according to the percentage of the time interval between two successive features. The corresponding positions were then interpolated within all of the other step cycles and these step cycles were then normalized according to the duration of each phase, resulting in 200 interpolated data points per step cycle. The normalized step cycles were displayed as a raster.
of traces and averaged to determine the mean ± SD of the EMG waveform at each point in the step cycle (e.g., Fig. 3B).

Walking segments during which stimuli were delivered were divided into normalized step cycles and the phase at which the stimulus pulse occurred was determined by its position between two adjacent step cycle inflections. Peristimulus records (~35 to 70 ms) were then arranged into rasters according to their phase order in the step cycle (note that they generally were not evenly distributed within a given phase as implied by the even spacing of the raster lines). Mean peristimulus records were computed from each step cycle phase (e.g., Figs. 4, C-F, and 7, A and B). In describing features of these reflex responses, I have followed the convention of grouping them into short-latency excitatory (P1, central delay ~1–3 ms), short-latency inhibitory (N1, central delay ~1–3 ms), and long latency excitatory (P2, central delay ~10–20 ms), as described previously (Abraham et al. 1985; Duysens and Loeb 1980; Loeb 1993; Loeb et al. 1987; Pratt et al. 1991). Some of these rasters contain small, brief peaks centered at zero peristimulus time, which represent stimulus artifact. The amplitude of the bin-integrated artifact (~time 0 in peristimulus rasters) and the evoked potentials in the sciatic nerve recordings were examined in similar rasters to verify that the stimulus efficacy did not fluctuate during the step cycle.

RESULTS

Many of the tendon transfers regrew completely or partially to restore normal anatomy, although this was often accompanied by hypotrophy of the originally modified muscles, sometimes with compensatory hypertrophy of unmodified synergists. Some of the spontaneously reconstructed anatomy was quite striking in its normal appearance, with complete reformation of the normal tendon paths of originally crossed muscles and only small amounts of diaphanous, yellow connective tissue around the original tendon sutures. In the case of animals with tendon transfers, the reconstruction sometimes required reforming ~1 cm of the original distal tendon that had been removed at the transfer surgery.

Ten of the 18 animals that originally underwent surgical alterations of their tendons had some persistent abnormalities of mechanical action. These were commonly not identical with the anatomic changes created at the original surgery; they are summarized in Table 1. Six of these were selected for detailed analysis (described later) on the basis of the quality of their locomotion, their electrophysiological data, and the opportunity to compare different outcomes involving the same muscles. Various records from some other animals had to be rejected because of technical problems with devices (particularly nerve cuffs, which sometimes slipped off the cutaneous nerves or had asymmetric thresholds or recruitment properties) and with locomotor behavior that sometimes was not sufficiently regular for reliable use of our phasing algorithm.

Cats with normal muscle actions (sham operation bilaterally or whose muscles regrew normally after tendon surgery) had symmetrical EMG patterns in the corresponding muscles of the two limbs during locomotion and cutaneous reflexes and paw-shaking (e.g., cat TT17, described later), although these patterns differed substantially among animals (as reported previously by Loeb 1993). Cats with persistent abnormalities in muscle action tended to have asymmetric cutaneous reflexes, as detailed below for stimulation of the superficial peroneal (Sp) nerve. Sural nerve reflexes in all animals were generally weaker, and patterns were less consistent. Analyses of symmetry therefore were not attempted. Ear-scratching and jumping behaviors were also too inconsistent for analysis, but paw-shaking behavior was regularly elicited and reasonably stereotyped in most animals.

Reorganization of flexor digitorum longus

In cat TT09, the flexor digitorum longus (FDL) and tibialis posterior (TP) tendons were crossed at age 18 days. At the time of device implantation (15 mo), the TP had regrown into its original insertion, although it was one-third smaller than the control muscle (Fig. 2). The crossed FDL muscle remained inserted into the TP tendon, but the origin appeared to have shifted onto the body of the popliteus (POP) muscle, which normally inserts adjacent to the FDL origin. The right (R)-FDL was substantially larger than the left (L)-FDL, consistent with integration of a substantial portion of the R-POP muscle mass. The change in origin of R-FDL resulted in a substantial knee-flexion moment and extended the range of motion of its fascicles. The FDL is much less pinnate than the TP, and it would therefore have had a greatly reduced range of sarcomere motion if it had acted only through the TP tendon. The R-FHL was somewhat hypertrophied, consistent with its role as the sole long toe flexor in the absence of FDL action on the toes.

Despite the substantial anatomic changes in cat TT09, the locomotor EMGs from all of its recorded muscles appeared to be symmetrical, as shown in the averaged records at the bottom of Fig. 3. Both FDL EMG electrodes were located on the midbody of the original FDL muscle, away from the fusion with R-POP. The detailed comparison of the EMG patterns of the FDL muscles shows them to be symmetrical (the mean envelopes of the two sides lie within each other’s standard

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<th>R</th>
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<td>0.44</td>
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<tr>
<td>FHL</td>
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<tr>
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<td>0.45</td>
</tr>
<tr>
<td>POP</td>
<td>1.03</td>
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FIG. 5. Cat TT17 anatomy. Transfer of FDL to TP was largely maintained but there was residual toe flexion from regrown connective tissue. R-FDL was small and R-FHL was large.
deviation at all phases), although this may be due in part to the predominance of stance-phase activity and the unusually small and inconsistent flexor burst in the left (normal) FDL. Although this is a normal variant, most animals have a larger or even predominant FDL burst just before and during footlift (Loeb 1993 and see Fig. 6). The TP itself tends to be active only during stance, but it is difficult to obtain high-quality EMGs from this small, deep muscle, and a comparison therefore was not attempted.

In contrast to its normal locomotor symmetry, the reflex

**FIG. 6.** Cat TT17 activity. A: treadmill locomotor activity was symmetrical in all muscles studied. Note typical flexor bursts at end-stance in both R-FDL and L-FDL muscles (arrows). B: relative recruitment and phasing of muscles in each leg was similar during paw-shaking in the swing phase of treadmill locomotion. Note gradual shift of FDL bursts from synchronous to antiphase with respect to TA and absence of participation by SO in both legs.

**FIG. 7.** Cat TT17 reflexes. Ipsilateral stimulation of Sp nerve at 2×T intensity produced similar P1-P2 reflexes around the time of the flexor burst in FDL in both legs.
EMGs in *cat TT09* provided one of the most striking examples of asymmetry. Figure 4, *A* and *B* shows well-matched evoked potentials obtained at twice threshold in both superficial peroneal nerves; Fig. 4, *C* and *D* shows the corresponding peri-stimulus rasters for the FDL. The left (intact) FDL had the typical short-latency excitatory reflex (P1) during the flexion phase, which was replaced by a strong inhibition of any background EMG activity in the right FDL, at both 2 T (Fig. 4D, open arrow indicates peak of corresponding excitatory reflex in the L-FDL) and 3 T Sp stimulus levels. A similar but less pronounced difference was seen in TP (not shown). The left FDL also had an inhibitory reflex during its stance phase activity in response to contralateral Sp stimulation, which was absent in the right FDL (not shown). Reflex patterns in the extensor digitorum longus (EDL) were also asymmetrical, even though it had undergone no surgical modification and appeared normal (Fig. 4, *E* and *F*). In particular, the usually strong P1 reflexes in early swing were absent or suppressed by

**FIG. 8.** Cat TT10. *A*: attempt to cross FDL with FHL resulted in complete atrophy of FHL and reduced size of FDL. *B*: during paw-shake, SO is normally suppressed, but it was phasically active on the surgical side (arrows), resulting in a stiffer and higher frequency movement. *C*: P1 reflexes during flexion phase in the intact FDL were largely absent on the surgical side (open arrow); mean of flexion phase peristimulus traces from complete raster (not shown).

<table>
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<th>wt. (g)</th>
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<tr>
<td>FHL</td>
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- **A**: Paw shakes
  - **LEFT**: SOL, LG, FDL, EDL
  - **RIGHT**: 0.1s

- **C**: Stim Sp 2xT
  - **L-FDL**: 30µV
  - **R-FDL**: 30µV
  - **F**: -40ms to 0ms
  - **Open Arrow**: +70ms
the strong N1 responses throughout the step cycle, which are
more typical of extensors such as SO and TP (Fig. 4F, open
arrows denote inhibition that appears to suppress background
activity as well as long latency excitatory reflexes apparent in
L-EDL during F and E1 phases).

In cat TT17, a simple transfer of the FDL to the TP resulted
in a substantial insertion of the FDL onto the TP tendon, but a
slip of connective tissue had regrown to the remaining FHL
tendon (normally common with FDL), resulting in a persistent
plantar flexion moment on the toes (Fig. 5). This modified FDL
had a normal origin but was severely hypotrophic, with com-
pensatory hypertrophy of the FHL muscle. The locomotor
EMGs appeared to be symmetrical (Fig. 6A), including a
prominent flexor burst at the beginning of stance in both FDL
muscles, which presumably contributes to digit plantar flexion
during early swing. The paw-shake EMGs also appeared to be
symmetrical (Fig. 6B). The cutaneous reflexes during locomo-
tion were symmetrical in their timing and phasing (Fig. 7); if
anything, they were stronger in the surgically modified R-FDL,
particularly the P1 responses around footlift (note calibration
bars and relative size of reflex compared with prestimulus
locomotor EMG activity).

In cat TT10, a tendon cross between FDL and FHL resulted in
essentially complete atrophy of FHL and a moderately
hypotrophic FDL (Fig. 8A). The paw-shaking behavior in this
animal was notably asymmetric, with the modified leg showing
a relatively low-amplitude, high-frequency oscillation accom-
panied by strong bursts in the SO (arrows in Fig. 8B). In the
contralateral, normal leg, the SO displayed its normal tendency
to be almost completely silent during paw-shaking, as de-
scribed previously (Smith et al. 1980) and confirmed in all
other animals in this series in which paw-shaking was ob-
tained. The locomotor patterns of the FDL and the unmodified
muscles were symmetrical (not shown), but the modified FDL
had a complete absence of the typically strong P1 reflex at
footlift seen in the intact FDL (Fig. 8C; open arrow over the
R-FDL’s mean peristimulus record corresponds to peak of P1
reflex in the L-FDL).

Reorganization of the EDL

The EDL normally has several somewhat distinct neuromus-
cular compartments with separate tendon slips that fuse to form
the common EDL tendon. In cat TT16, an attempt to create a
transfer of the R-EDL tendon to the peroneus longus (PL)
muscle resulted in a functionally split EDL muscle (Fig. 9A).
The superficial compartments (R-EDLs) remained attached to
the PL tendon, whereas the deeper compartments (R-EDLd)
were attached to a reconstituted EDL tendon. The total weight
of both portions of R-EDL was essentially identical with that of
the whole L-EDL. The R-PL was hypotrophic, but the com-
piled mass of R-PL and R-EDLs (the compartment with PL-
like mechanical action) was equal to that of the L-PL muscle.

EMG electrodes were placed over both the superficial and
depth regions of both R-EDL and L-EDL in cat TT16. The
locomotor and paw-shaking activity of both regions of these
muscles and the other ankle and long digit muscles were
symmetrical and unremarkable. The locomotor reflexes of
the L-EDL were small, whereas those for the L-PL were
strong, a variant pattern that is not in itself unusual. The two
functionally distinct portions of the R-EDL both had fairly
strong reflexes, but they were unlike each other or either the
L-EDL or L-PL in their phasing. R-EDLd (with EDL-like
mechanical action) had a strong P1 reflex in the flexion (F)
phase (arrow in Fig. 9B). R-EDLs (attached to PL) had no
P1 reflexes at all (open arrows; compare with peak reflexes
in the L-PL in corresponding phases) but had a strong P2
reflex in late stance (solid arrow in E3, unlike the L-PL).
The L-PL had a strong P2 reflex, but only during the F
phase, and a strong P1 reflex in E3.
Cat TT18 underwent essentially the same transfer as cat TT16, resulting in a similar split function, but the position of the compartments was reversed. The R-EDLd remained attached to the PL tendon, whereas the R-EDLs reattached to the EDL tendon (see Fig. 10A). Again, the total mass of both R-EDL compartments was approximately equal to that of the whole L-EDL. The R-PL was only mildly hypotrophic, and the combined mass of the R-PL and R-EDLd was 50% greater than that of the L-PL. Again, locomotor and paw-shaking EMG patterns were symmetrical in both portions of the EDL and the other muscles recorded. In this case, the excitatory reflexes in the left leg were generally quite prominent and were similar in latency and phasing for the L-PL and both portions of the L-EDL (see Fig. 10B). The reflexes in the R-PL were similarly brisk. The reflexes in both portions of the R-EDL were similar to each other but quite small.

Cat TT19 underwent the same transfer as both cat TT16 and cat TT18, but there was no restoration of any EDL tendon or mechanical action (see Fig. 11). The R-EDL muscle acted only on the ankle (as a dorsiflexor, everter, and abductor according to the normal insertion of the PL tendon); it was mildly hypotrophic. The peroneal muscles were all symmetrical, including the PL, but the right tibialis anterior (R-TA) was mildly hypertrophied. Interestingly, the combined mass of the three ankle dorsiflexors (EDL, PL, and TA) was identical (14.6 g) in each limb.

The locomotor activity of the R-EDL muscle (with insertion exclusively on the PL tendon) was typical of the EDL and identical with that of the normal L-EDL muscle, with a gradual onset of activity in flexion phase building to a maximum just before footfall (Fig. 12). The other ankle dorsiflexors in the left limb had typical activity, with a strong burst in the L-TA beginning just before footlift and peaking in early swing and low, irregular activity in the L-PL with small peaks in the early swing phase and early stance phase. By contrast, the normal R-TA had its activity displaced to late in the swing phase, similar to the altered R-EDL. The R-PL had a peak in early swing, similar to the normal L-TA, but its activity persisted into early stance, which is more typical of the PL. The EDL had weak reflexes in both limbs, but the PL and TA appeared to be asymmetric (Fig. 13). In summary, the R-PL and L-TA shared similar recruitment and reflex patterns, as did the L-PL and R-TA, as if they had been functionally crossed, although they were mechanically intact. Solid arrows in the R-PL raster denote excitatory reflexes whose counterparts appear in the intact L-TA; open arrows in the R-TA raster correspond to peaks of P1 reflexes during these phases in the L-TA.
DISCUSSION

Variability and learning

The objective of this study was to identify the nature and causes of interanimal variability that had been noted in locomotor and cutaneous reflex activity in normal cat hindlimb muscles (Loeb 1993). The methods used introduce other sources of variability that must be considered in interpreting these results:

1) Immature cats have a remarkable but somewhat unpredictable ability to restore normal musculoskeletal organization after surgical interventions.

2) Persistent unilateral changes in musculoskeletal organization give rise to asymmetries of muscle recruitment that may be idiosyncratic.

3) Individual animals with symmetrical limbs tend to have symmetrical locomotor and cutaneous reflex patterns but these differ idiosyncratically in their details between animals.

It is important to remember that the motor programs that developed and were displayed in these animals were grossly normal in their kinematic appearance and functionality for the animals. The asymmetries produced in this study were subtle, and their mechanical significance to the animals was not readily apparent. Most asymmetries occurred in reflex responses rather than unperturbed EMG patterns. This subtlety suggests that the contralateral control limbs were relatively unaffected by any mechanical or sensory abnormalities in the surgical limbs.

**FIG. 11.** Cat TT19 anatomy. Transfer of R-EDL to R-PL was maintained and complete, with no R-EDL function and only slight hypotrophy; PB and TA were somewhat larger in the surgical leg. Post mortem photographs show normal anatomy in sham-operation left leg. Proximal portion of R-EDL tendon (R-EDLt) inserted into distal end of PL tendon (R-PLt) at white arrow; there was no trace of the distal portion of R-EDLt on the dorsum of the foot (anterior view). Some of the EMG recording patches and leads are still visible.
Mechanical alterations of a muscle also affects proprioceptors in that muscle. Changes in background proprioceptive activity could result in changes of locomotor and/or cutaneous reflex activity of muscles through summation at interneurons and motoneurons without requiring changes in the CPG. Such a mechanism would be expected to produce more consistent changes in both locomotor and reflex patterns rather than the idiosyncratic changes noted here. For example, the mechanical effects of the incomplete EDL tendon transfers in cats TT16 and TT18 (Figs. 9 and 10, respectively) would have net similar effects on proprioception, but the changes in cutaneous reflexes were, in fact, quite different in the two animals (addition of extra reflexes in cat TT16 and loss of reflexes in cat TT18 compared with contralateral control). On the other hand, the severe atrophy of both the FDL and FHL muscles (and presumably their proprioceptors) in cat TT10 (Fig. 8) may account for the complete suppression of P1 cutaneous reflexes in the remaining hypotrophic FDL. The atypical SO activity during paw-shaking in this animal seems less likely to arise from such a sensory-gating mechanism, particularly given the normal participation of the FDL in the paw-shake.

The general paucity of effects on locomotor EMG may reflect a relatively high degree of genetically specified hardwiring in the locomotor CPG, or it may result from a high degree of learning that is constrained by simple biomechanics that are largely identical in all cats, even those with persistent but minor musculoskeletal abnormalities. For example, the exact phasing and balance of activity among the ankle dorsiflexors varies somewhat from animal to animal and may reflect a learned adjustment to balance the largely antagonistic moments produced by these muscles in the nonparasagittal axes (Young et al. 1993). This may account for the changes in the unmodified muscles in cat TT19 (Fig. 12). Considerably larger mechanical alterations than were attempted in these experiments (transposition of the LG and MG to the TA and removal of all natural ankle dorsiflexors) resulted in a significant limp and only subtle changes in the locomotor phasing of EMG activity (Forssberg and Svarten gren 1983). Cross-reinnervation of the adult cat’s FDL and SO also resulted in persistence of the recruitment patterns of the original motoneuron pools rather than the mechanical actions of their new muscles (O’Donovan et al. 1985). Similarly, rats do not seem to modify hindlimb motoneuron programs after either tendon or nerve transfers (Sperry 1945). An extensive study of adult polio patients after a variety of leg muscle transfers concluded that learning to switch muscle recruitment between swing and stance phase was rare, although not impossible, whereas more subtle reshaping of motor programs was common (Close and Todd 1959). Such results are more consistent with a CPG whose basic extension-flexion reciprocity is not capable of reorganization. By contrast, tendon transfers in cat forelimb resulted in functional recovery of locomotor and placing reflex kinematics; the strategies included both shifts in recruitment of the transferred muscles and compensatory changes in whole limb trajectory (Yumiya et al. 1979). Direct comparisons among species and limbs are complicated, however, by functional differences between plantigrade and digigrade locomotion and by the unknown nature and locus of the human bipedal CPG.

Substrates for plasticity

This study demonstrates that at least some of the details of locomotor programs are malleable rather than entirely genetically determined, but it does not indicate the timing or extent of the plasticity underlying the changes or their functionality, if
At the time of their tendon surgeries, these kittens were using only rudimentary crawling behavior that did not involve the foot placement, weight support, and pendular swing that are characteristics of adult gait. The fact that there were no asymmetries in animals that regrew their normal musculoskeletal anatomy suggests that either the learned patterns did not start to form until after this regrowth period, which presumably took at least a couple of weeks, or that any early learning was reversible. This begs the question of whether cutaneous reflexes can be modified in adult animals. If not, then presumably there is some “sensitive period” for plasticity.

If cutaneous reflexes can be modified by tendon transfers in reasonably mature animals, then it should be feasible to instrument the animals before the modification and to track the development of any changes. The surgery would be easier to perform and perhaps less prone to the dramatic restorations that occurred in many of the neonatal animals, but the tendon sutures would be more likely to fail before healing under the much higher stress applied by adult muscles and activities. Forssberg and Svartengren (1983) transferred gastrocnemius tendons to the TA in four adult cats and one kitten (3 wk) and immobilized the joints for several weeks. There was some
degree of regrowth in all, which was removed in a subsequent operation.

The use of better controlled behavioral paradigms coupled with more detailed measurements of muscle activity has started to reveal plasticity in various functions usually attributed to the spinal cord. Examples include behavioral conditioning of the H-reflex in monkeys (Wolpaw and Lee 1989) and rats (Chen and Wolpaw 1996), changes in proprioceptive effects on the spinal CPG after peripheral axotomy in cats (Whelan and Pearson 1997; Whelan et al. 1995), and changes in withdrawal reflexes after cutaneous nerve surgery in rats (Holmberg et al. 1997; McMahon and Wall 1989). In some cases the plasticity itself may reside in the circuits of the spinal cord; in others it probably results from changes in supraspinal circuits or in the effects of supraspinal influences on spinal circuits. Some of the reflex asymmetries reported in this study occurred at short enough latencies that they must reflect connectivity in oligosynaptic spinal circuits, but that connectivity may be influenced by tonic inputs from descending and spinal pattern generators. Motoneuron recordings in reduced preparations often reveal a wide variety of oligosynaptic excitatory postsynaptic currents (EPSPs) and inhibitory postsynaptic currents (IPSPs) after cutaneous nerve stimulation (Fleschman et al. 1984; Omeniuk et al. 1986; Pinter et al. 1982; Schmidt et al. 1988). These may reflect a substrate from which the command circuits normally build the coherent reflex patterns exhibited by whole muscles in intact animals (Fleschman et al. 1988). Unfortunately, this variability even within animals that matured normally suggests that it may be difficult to use such reduced preparations to identify the locus of change in animals with surgically induced asymmetries.

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