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Sequential Activation of Muscle Synergies During Locomotion in the Intact Cat as Revealed by Cluster Analysis and Direct Decomposition

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Krouchev, Nedialko, John F. Kalaska, and Trevor Drew. Sequential activation of muscle synergies during locomotion in the intact cat as revealed by cluster analysis and direct decomposition. J Neurophysiol 96: 1991–2010, 2006. First published July 5, 2006; doi:10.1152/jn.00241.2006. During goal-directed locomotion, descending signals from supraspinal structures act through spinal interneuron pathways to effect modifications of muscle activity that are appropriate to the task requirements. Recent studies using decomposition methods suggest that this control might be facilitated by activating synergies organized at the level of the spinal cord. However, it is difficult to directly relate these mathematically defined synergies to the patterns of electromyographic activity observed in the original recordings. To address this issue, we have used a novel cluster analysis to make a detailed study of the organization of the synergistic patterns of muscle activity observed in the fore- and hindlimb during treadmill locomotion. The results show that the activity of a large number of forelimb muscles (26 bursts of activity from 18 muscles) can be grouped into 11 clusters on the basis of synchronous co-activation. Nine (9/11) of these clusters defined muscle activity during the swing phase of locomotion; these clusters were distributed in a sequential manner and were related to discrete behavioral events. A comparison with the synergies identified by linear decomposition methods showed some striking similarities between the synergies identified by the different methods. In the hindlimb, a simpler organization was observed, and a sequential activation of muscles similar to that observed in the forelimb during swing was less clear. We suggest that this organization of synergistic muscles provides a means by which descending signals could provide the detailed control of different muscle groups that is necessary for the flexible control of multi-articular movements.

INTRODUCTION

Much interest has been expressed recently in the idea that the complex patterns of muscle activity that are observed during locomotion, scratching, and other multi-articular movements may be constructed from a small number of muscle synergies produced by a limited number of spinal modules (Bizzi et al. 2000; Grillner 1981; Grillner and Wallen 1985; Jordan 1991; Mussa-Ivaldi and Giszter 1992; Stein and Smith 1997; Tresch et al. 2002). This concept has been particularly well developed in the work of Bizzi and his collaborators, who have produced a series of studies over the last 15 yr suggesting that a wide range of behaviors can be produced by combining a very few muscle synergies (Bizzi et al. 1991; d’Avella et al. 2003; Kargo and Giszter 2000; Mussa-Ivaldi et al. 1994; Saltiel et al. 1998; Tresch et al. 1999, 2006). The approach in many of these studies has been to study a large number of different movements or patterns of muscle activity and to use mathematical techniques to determine the minimum number of synergies that are required to produce these movements. These techniques include principal component analysis (PCA) (Ivanenko et al. 2004, 2005; Patla 1985), independent component analysis (ICA) (Hart and Giszter 2004; Ivanenko et al. 2005); nonnegative least squares (NNLS) estimation (Saltiel et al. 2001; Tresch et al. 1999); and, more recently, nonnegative matrix factorization (NNMF) (Cheung et al. 2005; d’Avella and Bizzi 2005; d’Avella et al. 2003; Ivanenko et al. 2005; Ting and MacPherson 2005). In all of these methods, a data set containing multiple channels of electromyographic (EMG) activity from one or several behaviors is decomposed into a number of N-dimensional muscle synergies, where N is the number of EMG signals in the original data set. In most of the methods employed, each synergy activates all N muscles via a fixed series of weighting coefficients. Most studies using decomposition methods have concluded that a small number of muscle synergies (normally 3–6) is sufficient to produce a broad range of movements. The original EMG patterns are reproduced by multiplying each synergy by a time-varying command signal (in most methods, this is the component extracted by the decomposition) and then linearly combining them.

This has obvious implications for the neural control in that for any one given movement, the nervous system must produce a limited set of time-varying central command signals (corresponding to the number of synergies that were identified), each of which activates one of the synergies. Moreover, the fact that all muscles are activated by each synergy means that the central command signal predicted by some of the preceding methods is likely to be a complex waveform that bears little resemblance to the activity patterns of any of the individual muscles involved in the movement. In the case of locomotion, the input signal might be expected to vary continuously throughout the step cycle. However, in those studies that have examined the contribution of the motor cortex and the red nucleus to the control of intralimb coordination during locomotion (Armstrong and Drew 1984; Belozerova and Sirota 1993; Drew 1993; Drew et al. 1996; Lavoie and Drew 2002; Widajewicz et al. 1994), complex signals of this type, which vary continuously throughout the step cycle, are rarely seen in the discharge patterns of individual neurons. Rather most motor cortical neurons discharge in a phasic burst during only...
a restricted part of the step cycle. Indeed, in the studies from this laboratory examining the control of voluntary gait modifications, we have emphasized that different subpopulations of cortical and rubral neurons discharge only during a small part of the swing phase of locomotion. Inspection of these populations suggests that they discharge sequentially with some discharging at the onset of swing and others discharging progressively later in the swing phase (Lavoie and Drew 2002). Similarly, neurons in the motor cortex and red nucleus may also discharge sequentially to control intralimb coordination during reaching movements in cats and primates (Murphy et al. 1985; Van Kan and McCurdy 2001, 2002; Yakovenko and Drew 2005).

A similar argument may be made on the basis of recording of interneurons from the spinal cord during rhythmic behaviors. For example, both during fictive locomotion (Baev et al. 1979; Orlovsky and Feldman 1972) and fictive scratching (Baev et al. 1981; Berkinblit et al. 1978) in the cat, spinal interneurons discharge in brief phasic bursts of activity that are distributed throughout the gait or scratch cycle. A similar finding has also been made in the turtle during fictive scratching in which interneurons were found that were related to different phases of the scratch cycle as well as to muscle activity around different joints (Stein and Daniels-McQueen 2002).

During locomotion therefore the discharge activity of populations of neurons both in spinal and supraspinal regions is more compatible with a punctual control of a limited number of muscles, active at a specific time during the step cycle, than with a more continuous signal that would sum with other time-varying signals to determine the overall pattern of muscle activity throughout the step cycle. An alternative approach to that taken in the previous studies therefore is to suggest that the base synergies that produce locomotion are likewise discrete and active during only a part of the step cycle. In the present study, we perform an initial examination of this premise by making a detailed study of the temporal pattern of activity of muscles in the forelimb during treadmill locomotion by using a novel cluster analysis. This analysis differs from previous approaches using blind decomposition methods in that we have taken a more restricted definition of a synergy as comprising a group of muscles that are temporally co-activated and the period of activity of which begins and ends synchronously. The results show that small groups of muscle synergies are activated sequentially throughout the gait cycle, but particularly during the swing phase of locomotion, in a similar manner to that reported for neurons in the spinal cord, motor cortex and red nucleus.

Preliminary results have been presented in abstract form (Krouchev et al. 2003)

METHO DS

Data for these analyses were obtained from 10 cats that were chronically implanted with pairs of stainless steel electrodes to record EMG activity from selected fore- and hindlimb muscles in other studies (Bretzner and Drew 2005; Drew 1993; and Drew, unpublished observations). Data have been selected on the basis of the overall quality and quantity of the recordings from the different animals. In all of these experiments, the EMG activity was originally band-pass filtered between 100 Hz and 3 kHz (4th-order Butterworth) and amplified between 1,000 and 10,000 times to produce a signal of 1–2 V peak-to-peak. Data were digitized at a sampling frequency of 1 kHz. No further treatment of the signals was performed except where explicitly stated.

The EMG activity during locomotion was displayed on a monitor and interactive custom software was used to detect the onset and offset of distinct bursts of EMG activity (Fig. 1, A and B). Only periods of activity that were consistently observed in several cats were included in the analysis. A step cycle was defined as the time between two successive periods of activity in a flexor muscle that contributed to swing onset. In the forelimb, the step cycle was defined on the basis of the period of activity in the cleidobrachialis (CIB, protractor of the shoulder and flexor of the elbow) or of the brachialis (Br, flexor of the elbow); in the hindlimb, we used the activity of the anterior head of the sartorius (Srt, principally a flexor of the hip). The periods of activity in all other muscles were defined with respect to these standard muscles. Muscles active during swing were always associated with the burst of activity in the standard flexor muscle; muscles active during stance were always defined as being active after these flexor muscles became silent. Step cycle durations were normalized to unity, and the onset and offset of measured periods of EMG activity were expressed as a proportion of the normalized cycle with events occurring subsequent to the period of activity in the standard muscle being given positive values and those preceding it, negative values.

Data from any one continuous walking sequence, typically 20–40 step cycles, were graphed as a scatterplot in which the phase of the offset of the period of activity in a given muscle was plotted as a function of the phase of its onset (see e.g., Fig. 2C). This results in a set of points that defines the period of activity of the muscle (abbreviations for all muscles can be found in the legend to Fig. 1). In some cases, the activity patterns of muscles are co-extensive as is the case for the extensors, and for the second burst of activity in the teres major (TrM, retractor of the shoulder) and the extensor digitorum communis (EDC: dorsiflexor of the wrist and digits) in Fig. 1A. In other muscles, the activity patterns are temporally separate. Our general thesis is that muscles with bursts of activity that are co-extensive in time are functional synergists during locomotion and will act together to produce discrete elements of the overall behavioral strategy.

To determine objectively which muscles should be considered as functional synergists, we propose a novel clustering method and matching decomposition approach that is adapted to the particularities of the data set. Most cluster analysis methods are designed to work on an input set consisting of unclassified data points and to dissociate these data points into subsets along identifiable boundaries. The number and nature of the obtained data sets are frequently dependent on an a priori estimate of the number of clusters that is expected in the data. Such clustering techniques are inappropriate for our data set in which data points are already defined as belonging to a given muscle and must not be dissociated. We therefore developed an associative cluster analysis. The goal of this analysis was not to determine whether individual data points belong to one or another cluster (all data points of a given period of muscle activity must be treated as a unit) but whether the set of data points from different muscles overlap or not.

To this end, we first applied a variant of Rosner’s Test for detecting outlier data points (Rosner 1983), removing from the data set, for each muscle separately, those few observations that were farther from the mean than 2 SDs. After outlier removal for each muscle, we used a custom clustering algorithm to determine which muscles showed synchronous activation (see APPENDIX A). We then constructed an adjacency table across muscles. For each pair of muscles, we determined the distance between the centers of each cloud of data points. Data sets from different muscles that were separated by <1 SD were considered to be part of the same cluster. In cases in which muscles fulfilled the criteria to be part of more than one cluster, we used the adjacency table in a Matlab implementation of an algorithm by Knuth.
(Numerical Recipes, chapter 6.8) to determine the equivalence classes (Appendix A). We refer to muscles included in any one cluster as a synergy.

To facilitate comparison of the synergies determined on the basis of the cluster analysis with those obtained using other mathematical methods, we transformed each cluster into an asymmetric Gaussian basis function (see Fig. 10A) in which the peak value was placed midway between the mean of the onset of the period of activity in each cluster and the mean of the offset. The rate of decay of the Gaussian was calculated separately for each half of the waveform such that the two tails of the function (3 SD) occurred at the mean − SD of the onset of the burst and at the mean + SD of the offset of the burst. Because these waveforms are directly obtained from the cluster analysis, we refer to them as direct components.

For the factor analyses illustrated in Figs. 8–11, we used a two-stage threshold procedure to remove all base-line noise from the signal. The EMG data were then low-pass filtered using a zero-lag Butterworth filter with a cut-off at 15 Hz to produce signals (see Fig. 8A) similar to those used in previous locomotor studies (see Ivanenko et al. 2005). The resulting signals were normalized to a peak value of

FIG. 1. Example of electromyographic (EMG) recordings during locomotion from 2 cats, RS26 (A) and PCM2 (B). A: data are synchronized to the onset of activity in the elbow flexor, brachialis (Br) and records are shown for 3 other flexor muscles, the brachioradialis (BrR), the teres major (TrM) and the extensor digitorum communis (EDC) as well as 2 extensors, the lateral head of the triceps brachii (TriL) and the palmaris longus (PaL). ↑, onset of a measured burst; ↓, burst termination. A step cycle, in any given muscle, is defined as the period between 2 successive onsets of activity (↑) and is identified by the polygon below each trace. B: similar display for data recorded from PCM2, data in this case synchronized to the shoulder protractor and elbow flexor, cleidobrachialis (CIB). ⊗, muscle bursts that discharged together (see METHODS and below). C: illustrations showing the anatomical locations of the majority of the muscles that were used in this analysis (adapted from Drew and Rossignol 1987). The 3 leftmost illustrations show a lateral view of the limb while the rightmost illustration illustrates a medial view. ACD, acromiodeltoideus; Bic, biceps brachii; CIT, cleidotrapezius; ECR, extensor carpi radialis (brevis); ECU, extensor carpi ulnaris; LtD, latissimus dorsi; LvS, levator scapulae (ventralis); PrT, pronator teres; SpD, spinodeltoideus; SSp, supraspinatus; Tri, triceps brachii; long head; s, second.
FIG. 2.  A and B: periods of computer-rectified and averaged activity of 13 muscles simultaneously recorded from cat RS26: A, flexors; B, extensors. Data are synchronized with respect to the onset of Br (red vertical lines) and 2 complete step cycles are displayed (mean step cycle duration = 1,041 ms). Muscles in A and B are organized so that more proximal muscles are at the top. The TrM is repeated in B to serve as a comparison. Horizontal bars above each averaged trace indicate the mean + SD of the onset and offset of each burst of activity as measured from the individual cycles (see Fig. 1). Note that the averaging introduces a certain amount of temporal smearing in particular with respect to the second period of activity in muscles such as Bic and PtT. C: scatterplot of the time of onset vs. the time of offset of the phase of the activity of the different muscles showing the organization of the different periods of activity into clusters (see METHODS, Fig. 1 and APPENDIX A). Eight clusters are identified as 1–8 on the graph and in the legend. Colored circles illustrate the centers of each cluster (see APPENDIX A) and correspond to the colors used in the key that identifies the 8 clusters. Note that EDC and SpD frequently discharge twice in each step cycle but that the initial burst was not readily detectable in this cat; the bursts that we could measure are labeled EDC(2) and SpD(2). Outliers have been removed from this, and all other, scatterplots.
unity and then further treated by subtracting the mean value of the signal from each data point and dividing the results by its SD. This provided a data set that was compatible with that used by Ivanenko et al. (2005).

Principal components were calculated using the singular-value decomposition (SVD) function in Matlab. To allow direct comparison with the results of Ivanenko et al. (2004, 2005) the varimax rotation (Harman 1976; Kaiser 1974) of the initial principal components was computed using the corresponding Matlab function from the EEGLAB package (www.sccn.ucsd.edu/eeglab/) (Delorme and Makeig 2004).

Independent components were calculated using the infomax ICA routine (runica) (Bell and Sejnowski 1995) contained in the EEGLAB package. Comparison of the results from this method with those obtained using a different set of routines [FastICA algorithm (Hyvarinen 1999; Hyvärinen and Oja 2000)] showed that most components, and especially those explaining the most variance, were correlated with coefficients of determination ($R^2$) of $>0.9$.

Nonnegative matrix factorization (NNMF) of the data were computed using the Matlab implementation of Lee and Seung (1999). However, instead of using a fixed a priori user-specified number of iterations, we instead used a convergence criterion, continuing the iterations until there was no further decrease in the approximation error. The resulting components are neither orthogonal (as in ICA) nor independent (as in PCA) but have the advantage that both the components and the weighting coefficients have only positive values.

RESULTS

Forelimb activity patterns

The results of applying the cluster analysis to all of the periods of EMG activity recorded in a single animal are illustrated in Fig. 2 for cat RS26 in which we recorded EMGs from a total of 13 muscles in one forelimb. Three of these muscles exhibited two periods of activity in each step cycle providing a total of 16 periods of EMG activity (Fig. 2, A and B). Inspection of these data for the flexor muscles (Fig. 2A) shows that these muscles are not all activated simultaneously but that there is variation in the time in the step cycle when they become active as well as in their duration (and thus their offset). For example, activity in the TrM starts before the onset of activity in the Br, whereas the major period of activity in the EDC occurs after the end of the activity in the Br (see also Fig. 1A). In contrast, the activity patterns in the physiological extensor muscles, i.e., those active during the stance period (Fig. 2B), extensively overlapped.

The sequential nature of the activity in the flexor muscles is clearly seen in the two-dimensional plot of the data illustrated in Fig. 2C. Application of the cluster analysis defines six clusters in the swing phase (1–6) and two in the stance phase (7 and 8). The largest of these clusters (8) includes five extensor muscles while the next largest (cluster 6) includes four flexor muscles, all exhibiting a period of activity just prior to paw contact. We consider that each of the clusters containing more than one muscle defines a synergy. Clusters containing a single muscle whose activity pattern is distinct from other clusters identify putative synergies that might include muscles that were not recorded in these experiments (see DISCUSSION).

This characteristic temporal representation is constant across cats, as illustrated in Fig. 3 for the EMG activity recorded from cat PCM2 (illustrated in Fig. 1B). As for the example illustrated in Fig. 2C, there is sequential activation of, in order; the first period of activity in the TrM (cluster 2), the BrR (cluster 3), the Br (cluster 4), and the EDC (cluster 7). (NB. Cluster numbers in Fig. 3 do not necessarily correspond to those in Fig. 2C). In this example, however, data from additional muscles illustrate that there is an initial period of activity in the EDC and the ECR (cluster 1) that occurs before the TrM, and there is a second period of activity in the ECR (cluster 6) that occurs in an intermediate location between that of the Br and the EDC (see Fig. 1B). As such, Fig. 3 serves to emphasize further that there is an almost continual sequential activation of flexor muscles throughout the swing phase.

Although the relative temporal representation of the activity is similar in the two cats illustrated in Figs. 2 and 3, the absolute location of the clusters is variable. For example, the cluster containing the second period of activity in the EDC muscle (occurring in cluster 6 in Fig. 2C and cluster 7 in Fig. 3) occurs at different absolute locations in the phase plot. In cat RS26, the onset of this activity in most steps occurs at values between 0.2 and 0.3 (mean ± SD: 0.27 ± 0.08), whereas in cat PCM2, the onset often occurred at phase values of <0.2 (0.21 ± 0.03). This difference in the location on the phase plane simply reflects variability within the locomotor gait adopted in different cats; swing occupied a larger part of the overall step cycle in cat RS26 than it did in cat PCM2. Nevertheless, this variability makes it somewhat problematic to combine data from different cats to provide a global overview of the EMG activity in the limb.

To overcome this problem, we normalized the data from different cats by using linear least-squares estimation (LLSE) to adjust the phase of onset and offset of the periods of activity. This method provides a proportion coefficient that is applied to the muscle that we wish to map onto the reference data set. Essentially, a muscle that had a similar phase of activation to the muscle that we wish to add to the data base and that was

![Cluster Analysis](https://jneurophysiol.org/vol96/issue10/Fig3.png)

**Fig. 3.** Scatterplot of the activity patterns of 12 periods of EMG activity recorded from a different cat, PCM2. Data are organized in a similar manner to that of Fig. 2. Note that the colors and symbols used to identify clusters are the same as those in Fig. 2. However, because clusters are numbered sequentially within this data set they do not necessarily correspond to those in Fig. 2.
recorded in both data sets was selected as a reference. For example, Fig. 4 illustrates the method used to include the phase of activation of the ECR, recorded in cat PCM2, to the data set from cat RS26 that we used as our baseline example because of the variety of muscles that were recorded in that animal. In this example, we first used LLSE to determine a linear transform that shifted the phase of the second, principal, period of activity in the EDC (the reference muscle) recorded in cat PCM2 to the same value as that in cat RS26. This same proportion coefficient was then applied to the ECR. The phase of the activity in the ECR is displaced accordingly and therefore maintains the same relationship to the EDC as it had prior to the normalization. The averaged activity in the three muscles prior to normalization is shown in Fig. 4A and that after the normalization is shown in Fig. 4C. Figure 4B illustrates the process on the data from the individual step cycles. This process was applied to all additional periods of activity that were added to the data set of cat RS26. For example, the first period of activity in the EDC was referenced to the TrM as was the first period of activity in the latissimus dorsi (LtD), whereas the period of activity in the PaL was referenced to that of the TrM.

In the case of the CIB, we adjusted the phase of the end of the period of activity so that it maintained a constant relationship to the onset of the EDC(2) this took into account any variation in the duration of the swing phase. The results of this process are shown in Fig. 5, which illustrates the temporal representation of 26 bursts of EMG activity recorded from 18 muscles. Most of these periods of activity (16) are from cat RS26; the additional 10 periods of activity are taken from four other cats. This figure illustrates that the addition of the 10 extra periods of EMG activity adds an additional three clusters to those that were identified on the basis of the data from cat RS26 alone (Fig. 2C). One cluster includes the early periods of activity in the EDC and ECR (cluster 1) together with an early period of activity in the LtD muscle. A second is the result of the principal burst of activity in the ECR (ECR2), whereas the third is formed by the activity of the cleidobrachialis (CIB). Each of these three additional clusters was identified in cat PCM2, illustrated in Fig. 3. It is important to note that the normalization procedure did not influence the organization of the clusters but simply allowed the data from several cats to be displayed on a single illustration. This can be determined by comparing the relative location of each of these three additional clusters in Fig. 5 with those illustrated in Fig. 3. For example, the cluster containing ECR(1) and EDC(1) maintains its relative location with the TrM and the ECR(2) maintains its relative location with respect to the cluster containing EDC(2). It is also worth emphasizing that in three of the clusters (1, 9, and 11), the synergies include both proximal and distal muscles.

In general, the vector distance between the center of each cluster was in the order of 0.1–0.2. For example, the vector distance between clusters 1 and 2 was 0.10 and between clusters 2 and 3 was 0.13 (see Fig. S1 in the supplementary information). Other distances were greater. For example, the distances between clusters 5 and 6 was 0.13, whereas that between 8 and 9 was 0.17%.

From a behavioral point of view, these clusters correspond to different events in the locomotor cycle as illustrated both in Fig. 5, A and B. The first two clusters (1 and 2) are responsible for modifying the activity in the shoulder, wrist, and digit muscles that are responsible for unweighting and lifting the paw from the treadmill surface (lift leg). Subsequently, the muscles identified in clusters 3–5 act together to flex the elbow. At the same time, the muscles identified in clusters 6 and 7 act together to begin the forward transfer of the limb; the activation of these muscles continues throughout the entire period of the swing phase, ending just after the onset of activity in the extensor muscles. Following the elbow flexion, and as the limb is being transported forward, there is an activation of the ECR (cluster 8) that results in dorsiflexion.
of the paw. Subsequently, at the end of the swing phase, there is an activation of a number of muscles that prepare the paw for contact with the treadmill belt (cluster 9). Last, the extensors of the limb are activated during the stance phase of locomotion (clusters 10 and 11).

To determine the extent to which the clusters that we identified would be modified by small changes in the phase of activity of individual muscles or by experimental error in the detection of the onset and the offset of activity, we constructed an artificial data set based on the EMG activity patterns of the
eight muscles comprising the first three clusters in Fig. 5. Full details are provided in the supplementary information but, in brief, we generated a series of data sets in which the mean phase of the onset and offset of the activity of each muscle in each cluster was displaced by a value of ±1 SD of the variation in the cluster (defined in Appendix A). The onsets and offsets of each muscle (number of points identical to the original data set) were then generated using the SD of the original data set. The data were then reprocessed using the cluster analysis. We repeated this exercise for 100 repetitions for each of 10 changes in mean burst position. In the first group, the mean burst of all of the muscles was displaced by 0.0–0.1 of the maximum value (1 SD of the cluster), in the second group by 0.1–0.2, and so on to 0.9–1.0. This provided a total of 1,000 series of synthetic data. The results showed that the clusters were very stable to changes in mean activity of the muscles over the range tested and in >80% of the 600 trials for which the SD was displaced by ±0.6 SD, there was no change in the classification of any of the eight muscles in the three clusters.

**Hindlimb activity patterns**

We performed a similar analysis for EMG activity recorded from the hindlimb of the intact cat and obtained similar results to those for the forelimb. As illustrated in Fig. 6A, several muscles acting around different joints showed synchronous periods of activation. For example, one of the periods of activity in the semitendinous (St) occurred at the same time as a brief period of activity in the gastrocnemius lateralis (GL), whereas the other period of activity in the St occurred at the same time as a period of activity in the extensor digitorum brevis (EDB). The major periods of activity in the lateral (GL) and medial (GM) heads of the gastrocnemius also occurred at the same time during stance. These relationships can also be seen in the averaged activity of Fig. 6B that, in addition to the periods of activity in Fig. 6A, also shows synchronous activity in the tibialis anterior (TA) and the extensor digitorum longus (EDL) as well as in two other extensor muscles, the flexor digitorum longus (FDL) and the vastus lateralis (VL).

The results of the application of the cluster analysis to the nine muscles (13 periods of activity) obtained from the cat (MC23) used for the data of Fig. 6 as well as additional periods of activity from four other cats is illustrated in Fig. 7. The profiles of the clusters identified in the hindlimb show both similarities and differences with those observed in the forelimb. As for the forelimb, the initial period of activation is in the most distal muscles, such as the EDB, which becomes active just before the cat lifts the paw from the treadmill. Subsequently there is activation of the St muscle together with a burst of activity in the FDL and the gastrocnemius lateralis (GL), which, together, serve to flex the knee and raise the paw from the support surface. Flexion of the ankle (EDL) and transport of the limb [Srt and iliopsoas (Ip)] is followed by the braking action of the St (Wisleder et al. 1990) and by activation of the EDB in preparation for landing. Last, there is the almost simultaneous activation of a large number of extensor muscles during the stance phase of the step cycle. The major difference observed in the hindlimb was the smaller number of clusters during the swing phase. As a result the clear sequential representation of flexor muscles along the diagonal in Fig. 5 (i.e., clusters 1–5, +8, and 9) was less obvious in the hindlimb. Indeed, there were no muscle clusters in the hindlimb corresponding to clusters 3–5 and 8 in the forelimb, suggesting a simpler organization of the muscle activation patterns.

**Comparison with linear decomposition methods**

Most previous approaches to the question of whether a small, defined number of synergies are responsible for the production of the overall pattern of motor activity observed during locomotion have used blind decomposition methods. Our approach is clearly different from these, and the question arises as to the extent to which these two approaches identify similar or dissimilar sets of synergies. Therefore to provide a direct comparison of these results to those obtained by using blind decomposition techniques, we also performed PCA, ICA, and NNMRF analyses on our data set from the forelimb.

As a first step, all of the averaged EMG traces used to create Fig. 5 were filtered to remove high-frequency noise as illustrates in Fig. 8A for a subset of the muscles. Application of PCA to this signal showed that 90.5% of the variance in the original signals could be explained by the first five components (Fig. 8, B and C) with the first component alone accounting for almost half of the variance. Following the method of Ivanenko et al. (2004), we then applied varimax rotation to these signals which had the effect of reducing the amount of the variance.
explained by the first component (Fig. 8, D and E) and also of producing more phasic components that better resembled the original EMG patterns. As in the studies of Ivanenko et al. (2004, 2005), we retained only the five components with corresponding eigen values of >0.5. The first four of these components were maximally active just before and during the swing phase, whereas the other was active primarily during stance. Figure 8F shows the five muscle synergies that are associated with these components. Inspection of the weighting coefficients shows that each synergy facilitates activity in some of the 18 muscles in the original data set and suppresses it in others. In addition, inspection of this figures shows that most
Muscles are facilitated by some synergies and suppressed by others, although each synergy maximally influences different groups of muscles. As explained in the Introduction, the original pattern of EMG activity can be reproduced by multiplying each of the time-varying components by the weighting coefficients in its associated synergy and then linearly combining these signals.

Application of ICA to these same EMG data resulted, by definition, in a series of 18 components (1 for each input signal) with each of the first 7 components supplying >5% of
variation of the original signal for a total of 72.1% of the variance (Fig. 9). A total of 9 components were required to explain 80% of the variance and 13 to explain 90%. Inspection of the components illustrated in Fig. 9 shows that the peaks of the waveforms generated by the ICA were distributed sequentially throughout the step cycle, albeit with most of them occurring in the first 40% of the step cycle, corresponding to the swing phase. This resembles the properties of the clusters identified in Fig. 5, most of which are active for only a short period of the step cycle, mostly during the swing phase.

To determine quantitatively the extent to which the results obtained from the cluster analysis and from the ICA resembled each other, we performed a reiterative correlation analysis between the waveform of each direct component (Fig. 10A; see METHODS) and the waveform of the 11 independent components that explained the greatest variance. The results from this analysis are shown in Fig. 10B in which the independent components best correlated to the direct components representing each cluster are illustrated. For example, the best correlation with the direct component derived from cluster 1 illustrated in Fig. 5A was obtained with independent component 10 (IC10) identified from the ICA. The coefficient of correlation \( r \) was 0.95 although this independent component contributed only 3.9% of the total variance of the original data set (see Fig. 9). Overall, the coefficients of correlation varied from a low value of \(-0.33\) (indicating the best correlation was with the inverted component) to a high value of 0.95. Nine of 11 correlations had values of \( r > 0.5 \), indicating that there was a very close overlap in the phase of many of the direct and the independent components. However, because the duration of the components obtained from the ICA was very brief, the correlations with the direct components corresponding to the extensor synergies (DC10 and DC11) were weak. Figure 10C illustrates the weighting coefficients of the muscle synergies associated with each of the independent components illustrated in Fig. 10B. As for the weighting coefficients derived from the varimax rotation (Fig. 8F), the synergies produced by the ICA all provided substantial positive or negative weighting factors to multiple muscles.

In a complementary analysis, instead of correlating the two series of components, we correlated the weighting coefficients of the synergies derived from the components. For this analysis, the muscle activation patterns associated with the direct components were correlated separately with the positive and negative weights from the matrix produced by the ICA. The results from this analysis equally emphasize the similarities between the synergies identified by the two methods. For example, the muscle activation patterns associated with DC2 were best correlated with the muscle synergy associated with IC11, which is active at the onset of the swing phase, i.e., at the same time as the cluster from which DC2 is derived. The independent components associated with the synergies best correlated with the muscle activation patterns of DC3–DC7 were sequentially activated just after the onset of swing. The phase of activation of these ICs was similar to that of the direct components, DC3–DC7 (compare with Fig. 10A).

In summary, similar correlations were observed whether we correlated the components and inspected the associated synergies or whether we correlated the weighting coefficients associated with the synergies and inspected the waveforms of the components. Indeed, this comparison suggests that the major difference in the muscle activation patterns or synergies identified by the two methods is that the synergies identified by the cluster analysis include fewer muscles than those identified by ICA. In other words, there is a sparser representation in the synergies identified by the cluster analysis.

Because the ICA produces synergies containing weighting coefficients with both positive and negative weights, whereas the synergies derived from the cluster analysis can only be represented by positive weights, we also compared the results from the cluster analysis with those obtained by NNMF. For this analysis, we constrained the NNMF algorithm to produce 11 nonnegative components, the same as the number of clusters that we obtained. This approach is different from that normally used (Cheung et al. 2005; Ivanenko et al. 2005; Lee and Seung 1999) in which the goal of the algorithm is to determine the smallest number of components that can explain the largest variance. The results, illustrated in Fig. 11, are very similar to those obtained from the ICA analysis. First, as for the ICA, the NNMF analysis provides a series of phasic waveforms that were active during relatively discrete times in the step cycle. Second, correlating the muscle activation patterns derived from the cluster analysis with the weighting coefficients associated with the synergies derived from the NNMF analysis resulted in the NNMF-derived waveforms being organized in a very similar sequential order (Fig. 11C) to those obtained from the cluster analysis and illustrated in Fig. 10A. Moreover, in contrast to the ICA, NNMF also provides a good

![ICA](image-url)
representation of the extensor synergies as can be observed by comparing component NNC3 and NNC1 in Fig. 11C with components DC10 and DC11 in Fig. 10A. As for the ICA, the major difference between the muscle activation patterns obtained from the cluster analysis and those obtained from the purely mathematical analysis lies in the sparser nature of the former. However, because the NNMF analysis is limited to positive values, the number of muscles with strong weighting coefficients in any given synergy is more limited than those obtained by either the PCA or the ICA.
**DISCUSSION**

The results from this study show that small clusters of muscles (synergies), in both the fore- and hindlimb, are activated sequentially during locomotion. This sequential activation was clearest in the swing phase of the forelimb for which we identified nine clusters. As illustrated in Fig. 5, each of these nine clusters was related to a discrete number of behavioral epochs during the step cycle with those clusters occurring at the onset of swing being responsible for lifting the limb from the ground and those active at the end of swing being responsible for preparing the paw for contact with the support surface. A similar sequential activation was observed in the hindlimb although, overall, fewer synergies defined the swing phase of this limb. We suggest that these synergies provide a flexible means by which descending systems can regulate and modify limb activity.

**General considerations and methodology**

The number of clusters identified depends on a number of factors, and we have to address the extent to which these clusters represent a true and stable feature of the step cycle. For example, changing the values of the variables in the algorithms used to identify outliers or to include points within a cluster will increase or decrease the number of clusters. Determining the values to use in these algorithms is subjective, but we believe that the values that we used for both of these parameters result in a justifiable organization of the data. For example, removing outliers that are > 2 SD from the mean value ensures that the clusters are influenced neither by including spurious data points nor by removing the innate variability of the muscle activity patterns from the data set. Decreasing the limits for inclusion of muscles into a cluster results in the division of the major synergy defining the activity pattern of the extensor muscles during stance into multiple clusters. Such a division seems unjustified on the basis of the overall similarity in their activity patterns and their common function during locomotion (see e.g., Fig. 2). Conversely, increasing the limits for inclusion results in a number of synergies being combined. For example, the TrM and the PrT would be included in a single cluster despite the clear differences in the phase of their onset and offset, as illustrated in Figs. 1–3. The validity of the clusters that we identified is also suggested by the creation of the synthetic data sets that we created (see supplementary data). This showed that the clusters that we identified are resistant to relatively large changes in the mean phase of the individual EMGs included in these clusters. Nonetheless, it is possible that, in a very few cases, some muscles that were placed into different clusters (e.g., Br and BrR) might be misclassified. However, even if the clusters containing these two muscles were merged, the resulting cluster would only contain three muscles (combining clusters 3 and 4 in Fig. 5A). More importantly, merging two adjacent clusters would never produce a single synergy containing muscles active at quite different phases of the step cycle, e.g., Br and EDC. Moreover, it should be emphasized that the sequential organization illustrated in Fig. 5, with essentially the same order, was observed in all of the cats to which we applied this analysis. As such we believe that the identification of a number of sequentially activated synergies illustrated by the application of this cluster analysis provides an accurate description of the pattern of EMG activity produced during locomotion.

Another issue that has to be addressed is whether recordings from additional muscles of the forelimb would produce additional clusters or modify those that we have already identified. We believe that this is unlikely for two reasons. First, all of the clusters that we identified are associated with clearly identified kinematic and behavioral features of the step cycle, e.g., paw lift, wrist dorsiflexion during mid-swing, limb transport etc. There is little likelihood that additional muscles would identify additional clusters or behavioral epochs. Second, in combining the muscles from different cats into the single synthesis illustrated in Fig. 5, we found that all additional muscles recorded from other animals fit into the clusters identified on the basis of the data from cats RS26 (Fig. 2) and PCM2 (Fig. 3). We believe that additional recordings would primarily modify the number of muscles in a given cluster rather than modifying the number of clusters.

In this analysis, we propose that all of the muscles included in a single cluster should be considered a synergy in much the same way that the blind decomposition methods identify synergies (see following section). In this respect, the question of what constitutes a synergy is always controversial. Without listing all possible definitions, synergies are normally understood to represent groups of muscles that act together to perform a particular function (Lee 1984; MacPherson 1991). Our definition of a synergy, as comprising a group of muscles that are temporally co-activated and the period of activity of which begins and ends synchronously, falls within this general definition but is clearly restricted. By this working definition, some synergies defined as being distinct in this study may be viewed as forming a single synergy by others. For example, we have defined two synergies (defined by clusters 1 and 2 in both Figs. 3 and 5) as being involved in lifting the paw from the treadmill belt even though both of these synergies may be considered as contributing to a single overall goal. Clearly a neural mechanism is required to allow the production and

**Fig. 10.** A: 11 clusters identified in Fig. 5A have been transformed into Gaussian waveforms that we refer to as direct components (DCs). Data are synchronized to the onset of activity in the Br, and 1.5 full step cycles are illustrated. The components are organized, from top to bottom, according to their time of onset, as in Fig. 5A. B: components identified from the ICA analysis (Fig. 9) are displayed according to their degree of correlation with the waveforms illustrated in Fig. 10A. Values to the left of the figure indicate the coefficient of correlation (r) of each pair of waveforms together with the original order of the ICs in Fig. 9; negative values indicate inverted waveforms. C: weighting coefficients associated with the components illustrated in Fig. 10B. It should also be noted that any given IC could be best related to > 1 of the DCs. Thus IC5 is the best correlation with both DC5 and DC6. Only the 11 ICs illustrated in Fig. 9 were used in this correlation analysis. D: muscle activation patterns identified on the basis of the cluster analysis are placed in the same order as in Fig. 10A. E: weighting coefficients identified from the ICA that are best correlated with the muscle activation patterns shown in Fig. 10D. Note that these weighting coefficients have been autoscaled. F: components associated with the weighting coefficients illustrated in Fig. 10E. Note that for all except 1 of the comparisons shown in Fig. 10E, the best correlations were always found with the positive loadings, the exception being that between DC11 and IC2. As for the correlation in A–C, each of the synergies from the IC could be correlated to > 1 of the synergies derived from the cluster analysis.
independent control of these two sequential synergies. Although the mathematical decomposition methods do provide a solution to this problem, it is complex, and we would argue that the phasic periods of activity observed in the discharge patterns of motor cortical and rubral neurons (Drew 1993; Lavoie and Drew 2002) provide a more parsimonious explanation of how the synergies defined by clusters 1 and 2 would be controlled independently. Certainly a future goal must be to test this hypothesis more directly.

Questions may also be raised concerning muscles that have similar phases of onset but the activation of which lasts for different periods of time (e.g., the ClB and the Br). Such muscles in one sense are certainly synergists in that they both contribute to the flexion of the elbow. However, our analysis places them in different clusters because of the differences in the timing of the end of the period of activity. We propose that this is valid given that elbow flexion (to which Br and ClB contribute) can be modified independently of shoulder protraction (to which only ClB contributes). For example, our previous studies have shown that stepping over a short, wide obstacle preferentially increases activity in ClB with a smaller effect on Br, whereas stepping over a tall, narrow obstacle has the inverse effect (Drew 1988, 1991). Considering these muscles to belong to different synergies is compatible with the observed results during stepping over obstacles of different dimensions.

A related issue is the fact that some of our synergies consist of only one muscle; the ECR (cluster 8 in Fig. 5) being an example. Considering a single muscle to be a synergy is clearly a misnomer. However, we retain the term because it is compatible with the concepts underlying this work and because the time of activation of this muscle is compatible with it having a contribution to the quite discrete dorsiflexion of the paw that occurs in mid-swing. In addition, identifying the ECR as a synergy emphasizes that it is activated independently of muscles included in other synergies that are activated before and after this muscle becomes active. Moreover, the fact that we have identified neurons that discharge equally punctually in phase with this period of activation (Drew 1993) strongly suggests that it is an event that is subject to a control signal independent of that contributing to the activity and modification of other synergies. Last, it is highly likely that other wrist dorsiflexor muscles (not recorded in our studies) discharge at a similar time. As such, we consider that the
ECR “synergy” is representative of an overall behavioral control strategy in which small groups of muscles are activated simultaneously and in which different groups of synergistic muscles are sequentially activated throughout the swing phase.

Comparison with other methods

As we have stated earlier, most previous approaches to the identification of synergies during locomotion have used blind decomposition methods. It was, therefore, important to determine the extent to which the results obtained from these methods compare with those obtained using our cluster analysis. The results from this comparison, performed on identical data sets, showed two major differences between the two approaches. First, in this study, we identified 11 synergies for the forelimb, whereas the results from methods such as PCA normally show that between three and six synergies are sufficient to explain between 80 and 90% of the variance in most motor behaviors that have been studied (Ivanenko et al. 2004). Second, each of our synergies is defined by the limited number of muscles included in that synergy and by the fact that each synergy can, potentially, be activated independently of any other. The decomposition approaches always yield synergies comprising all of the muscles under study. These differences are inherent in the different methodologies that are used. Whereas in this study muscles must be simultaneously active to be considered as synergists, other methods identify muscles the activity of which covaries in different behaviors, regardless of the temporal relationship between them. Clearly both definitions of a synergy are valid, but each has different implications concerning the types of descending signals required to activate or modify these synergies.

The synergies identified by the decomposition methods normally comprise all of the muscles included in the analysis with a weighting matrix determining which muscles in a given synergy are activated strongly and which weakly. The overall motor pattern at any given moment is then obtained by multiplying each component by its corresponding weighting matrix and then linearly summing together the resulting waveforms. As such, the descending signals to control any given movement must activate all synergies simultaneously. In contrast, the synergies identified by our cluster analyses can be activated independently by phasic signals active only during the time that the particular synergy is active. This latter interpretation is more compatible with the discharge patterns of motor cortical neurons during locomotion, many of which discharge phasically for relatively brief periods during the swing phase (Drew 1993).

Although there are conceptual differences in approach and interpretation, there are, nonetheless, similarities in the results obtained from the cluster analysis and the results from the factor analyses performed in this study and by others. For example, Ivanenko et al. (2004, 2005) have performed detailed studies of human locomotion using PCA and have shown that five components can explain \( \leq 90\% \) of the variance in EMG activity patterns recorded under a variety of conditions. These components occurred at different times during the gait cycle, and some at least were suggested to be related to activation of muscle patterns at distinct times of the step cycle. In the forelimb, our PC analysis also showed that a limited number of varimax components (5) could explain 90% of the variance. This indicates a strong similarity in the underlying patterns of EMG activation in the cat and the human, although it should be remembered that our factor analysis was performed on forelimb activity. However, as illustrated in Fig. 8, each of the synergies associated with the principal components activated multiple muscles, both with positive and negative weights. This is quite different from the sparse representation of the synergies indicated by our cluster analysis.

The results obtained by using ICA and NNMF bear some quite striking similarities to those obtained with the cluster analysis. For example, both the ICA and, more particularly, the NNMF analyses identify components that are phasically active during restricted periods of the step cycle and strongly correlated with the direct components identified by the cluster analysis. Moreover, the correlations between the weighting coefficients of the synergies obtained by ICA and NNMF with those identified on the basis of our cluster analysis showed equally strong similarities in the composition of muscles within the synergies defined by the two methods. This was particularly striking for the NNMF analysis in which the weighting coefficients are constrained to positive values (Fig. 11). Indeed, if one considers only the largest and, in the case of the ICA, only the positive, weighting coefficients from the ICA and NNMF analysis, then as for synergies defined by the cluster analysis, each synergy influences primarily, a limited set of muscles. This is similar to the results presented by Hart and Giszter (2004) in their study of motor behaviors in the frog in which ICA was used.

The similarities in the results from using these different methods, at least when comparing only the muscles with the largest weighting coefficients, suggests that all of these approaches extract comparable synergies from the overall pattern of locomotor activity. This is similar to the results obtained in the recent study by Tresch et al. (2006) in which different blind decomposition methods gave comparable results when tested on both simulated and real experimental data sets. Whether we would obtain similar results between our cluster analysis and blind decomposition methods from more complex and diverse behaviors remains to be determined.

Comparison with the hindlimb and implications for a modular organization

The major goal of our analysis was to determine the synergies underlying the control of the forelimb. However, for comparative purposes, we also applied the same cluster analysis to the hindlimb. This analysis identified only 7 synergies compared with 11 in the forelimb. Moreover, the clear sequential activation of forelimb muscles observed in Fig. 5 was not as prominent in the plots of the hindlimb activity of Fig. 7. Nevertheless, the data do indicate that there is a complex pattern of organization in the hindlimb locomotor pattern with five of the synergies occurring during the swing phase of locomotion. Although fewer than in the forelimb, this does speak to a complex pattern of organization in the generation of the hindlimb pattern.

The smaller number of synergies that we identified in the hindlimb might reflect the smaller number of muscles that we
recorded from this limb. However, we feel that this is unlikely as inspection of several comprehensive studies of the activity patterns of hindlimb muscles (Prochazka et al. 1989; Rossignol 1996; Yakovenko et al. 2002) reveals no muscles whose onset and offset times would fill the empty phase space in Fig. 7. Moreover, the organization revealed by our cluster analysis also resembles the results from the analysis of hindlimb activity performed by Yakovenko et al. (2002). For example, they also emphasized simultaneous activation of the St, SMA and FDL at foot lift (our cluster 2 in Fig. 7) and referred to this group as retractors. Similarly, the major flexor and extensor muscles are divided into two groups in both analyses. Our results extend their analysis by identifying separate clusters active at paw lift and paw contact. The latter group is clear in the average profiles of the muscles illustrated in their study but was not included as a separate group in their simulation. As in our study, Yankovenko et al. (2002) found no evidence for any extensive sequential organization of hindlimb muscles during the swing phase of locomotion. As such, we feel that the difference in the number and organization of the synergies found in the forelimbs and the hindlimbs reflects a real difference in the control of the two limbs. The major function of the hindlimb is in propulsion of the animal, and this function can be produced with relatively simple patterns of muscle activation. In contrast, the forelimb has evolved to be also used in more complex movements, such as reaching, grasping and prey capture. It is probable that the more differentiated pattern observed in the forelimb reflects the more complicated anatomy of the forelimb and the additional requirement for fractional control of muscle groups. In particular, the shoulder of the cat, which is attached to a floating scapula, has more possible degrees of freedom (English 1978) than does the hip, which is attached to a rigid pelvis. In addition, the wrist is more mobile than the ankle and is capable of large degrees of pronation and supination in addition to flexion and extension; in addition, it also has a larger capacity for lateral deviation than the ankle (inversion and eversion). The fact that there is some pronation of the wrist during locomotion, and that the paw needs to be dorsiflexed in preparation for contact with the ground, probably also contributes to the more complicated pattern in the forelimb. Nevertheless, it should be noted that EMG activity patterns acting around the hip, knee, and ankle are differentially modified during steps over obstacles (Widajewicz et al. 1994), and it is possible that greater complexity in the synergies required to produce hindlimb swing might be seen during gait modifications.

The synergies that we identified were based on EMG patterns recorded in the intact cat. As such, it is likely that the final expression of the synergies that we have identified is the result of the combined action of central spinal circuits together with input signals from descending pathways and from peripheral afferents. In the intact animal, it is clearly impossible to separate these three control signals as they are all essential for the production of the complex patterns of activity that we recorded. Nonetheless, we believe that our results also have implications for the mechanisms underlying the central generation of the locomotor rhythm. For example, there is ample information to suggest that the general activity patterns observed in the hindlimb during locomotion in the chronic spinal cat (Barbeau and Rossignol 1987; Bélanger et al. 1996) as well as during fictive locomotion (in the absence of rhythmic peripheral afferent input) (see Rossignol 1996) resemble those observed in the intact animal.

The formal and objective identification of temporal synergies in the hindlimb that we have made in this study are in agreement with the modular organization of the spinal cord suggested by several authors (Berkowitz and Stein 1994; Grillner 1981; Grillner and Wallen 1985; Jordan 1991; Lafreniere-Roula and McCrea 2005; Stein 2005; Stein and Daniels-McQueen 2002; Stein and Smith 1997). In most of these models, it is suggested that select groups of motoneurons or muscles with a similar function are activated simultaneously with the modular organization providing an opportunity for reorganization of the module according to the specific behavior being produced. Direct experimental evidence for such a modular organization has come particularly from work in the turtle in which interneurons in the lumbar spinal cord have been found to discharge in phase with the activity of nerves innervating either the flexor or extensor muscles at the hip joint during fictive scratching (Stein and Daniels-McQueen 2002). The authors made the important observation that activity in hip flexor interneurons was seen even if the hip extensors were silent. Moreover, the fact that interneurons in the spinal cord of fictively walking cats discharge in discrete bursts at restricted times during the step cycle (Baev et al. 1979; Orlovsky and Feldman 1972) is also in agreement with the idea of multiple synergies proposed in this work.

The results from our study are equally compatible with a modular organization for the generation of the forelimb rhythm. However, the number of synergies identified in the forelimb suggest that the modular organization is more complex than in the hindlimb. In particular, the clear, sequential organization of the pattern of activity in the flexor muscles throughout the swing phase suggests the existence of modules in which the termination of activity in one module might be responsible for the activation of the next in the sequence. Moreover in both the hindlimb and the forelimb, several synergies activate muscles acting around different joints; this is especially clear in the forelimb for the cluster of muscles that are active just prior to paw placement. This suggests either that the modules are organized so that the synergies, or at least some of them, are hardwired according to function (see e.g., Lundberg et al. 1987) or that a larger number of smaller modules can be combined according to context. Last, in many cases, muscles acting around the same joint (e.g., Pte, ECR and EDC) are active at different times throughout the swing phase. This also speaks for a modular organization based on functional multi-joint synergies but one in which individual muscles may be represented more than once.

**Functional implications**

This study has identified synergies that are active at different times during the step cycle. Each synergy is composed of a small number of simultaneously active muscles. We suggest that the activity of the muscles in each synergy is regulated by discrete phasic command signals from descending systems. This suggestion is in agreement with the findings that many pyramidal tract neuron (PTNs) in the cat motor cortex also discharge in discrete phasic bursts of activity during the step
cycle and that different populations of PTNs are active during different parts of the swing phase. One of the implications of this suggestion is that individual motor cortical neurons would act to modify the activity of synergistic muscles acting around different joints. Changes in neuronal discharge frequency would be expected to modulate the magnitude of the muscle activity, whereas changes in the time of activation of these neurons would cause changes in the relative phase of activity of the different synergies. The fact that some synergies included muscles acting both proximally and distally is in agreement with insights into cortical organization obtained from microstimulation in cats and primates and from spike-triggered averaging (STA) studies in primates. For example, motor cortical microstimulation in the intact cat frequently activates muscles acting around both distal and proximal joints (Armstrong and Drew 1985; Drew 1993). Similarly, a study by McKiernan et al. (1998) using STA of motor cortical cell in the primate showed that 45.5% of the cortical cells produced post spike facilitation in both proximal and distal muscles.

In this study, we have restricted our analysis to the temporal characteristics of the synergies that underlie the production of the base locomotor rhythm. The identification of a large number of temporally distinct synergies, even during such a simple behavior, provides a flexible substrate that could be used to produce a wide range of motor behaviors. Indeed, a major question that is raised by these results is the extent to which the synergies identified during unobstructed treadmill locomotion are conserved in different conditions. For example, we have previously shown that when cats modify their gait to step over obstacles, there is a change in the amplitude, duration, and relative timing of the EMG activity patterns of many muscles in both the forelimb and hindlimb (Drew 1993; Widajewicz et al. 1994). We expect that most synergies will be conserved during these activities and that the amplitude of the level of activity in muscles identified as synergists would be modified in a similar, proportional manner. Moreover, the relative phase of activation of muscles identified as synergists would change according to the size and shape of the obstacle. We propose that these modifications are produced by the changes in the discharge frequency and phase of activation of sub-populations of PTNs that we have observed (Drew 1993; Drew et al. 1996).

We further suggest that these same synergies would be conserved across different behaviors. For example, it has been suggested that the neural mechanisms underlying the control of reaching movements evolved from those that control voluntary gait modifications (Georgopoulos and Grillner 1989). As such, one may expect that the synergies identified during locomotion will also be used during reaching, although the phase of activation of different synergistic groups will likely vary. These propositions are compatible with the results obtained in the recent studies of d’Avella and Bizzi (2005) and Ivanenko et al. (2005), by using decomposition methods, that some synergies are maintained across behaviors and others are modified or introduced. It remains to be determined whether changes in the organization of these synergies are reflected in appropriate changes in the discharge patterns of cells in the motor cortex and red nucleus.

**APPENDIX A: SPECIALIZED ALGORITHM FOR CLUSTER ANALYSIS**

Each EMG burst is represented by only 2 values, the relative phases of the onset and the offset. A simplified view of the sampled data for a single muscle is based on the marginal sample means (Ms, Ms) and SDs (Sx, Sy) of the onsets (x) and offsets (y) (Fig. A1A). The vector, R(i) is a compact representation of Sx, Sy for burst i (Fig. A1A).

Two sets, i and j of points (x,y), sampled from two different EMG bursts, are considered to be adjacent (neighbors) if the distance between the mean points of the two scatter clouds is less than Frac times the sum of the SD. Let d denote the vector difference between the centers (averages) of the scatter clouds. Then for each (kth) EMG in the pair compute the normalized projections p(k) of R(i) onto the direction spanned by d (Fig. A1B)

\[ p(k) = c(Frac \cdot d), k = i,j \]

where the actual projection, \( \sigma \) is computed using the inner vector product

\[ \sigma = R(k)^T \cdot d = Sx,dy + Sx,dl, \]

Two bursts are then considered adjacent if

\[ p(i) + p(j) > d \]  

Figure A1B presents schematically the overlap identification process. Note that for the pair of bursts represented by the green and red bounding rectangles the inner vector products are less than the vector difference and the two sets do not overlap. In contrast, there is a clear overlap of the bursts represented by the blue and the red bounding rectangles; these bursts are, therefore considered to be part of the same cluster.

Figure A1C presents the overlap identification on a subset of the real EMG data. This example is taken from the RS26 data used for computing the clusters illustrated in Fig. 2C. Inspection of the vectors in this figure shows that the adjacency criterion (Eq. A1) is met for the pairs TrM/PtT, PrT/BrR, and BrR/Brc. The question then is, if burst i is adjacent to burst j, and burst j is adjacent to burst k, are i and k neighbors and should i, j, k be considered as a cluster?

To address this question, we applied a correction for nontransitive neighborhoods in which the adjacency table was validated for triplets

\[ (i,j,k), \text{ where } i < j < k \]

In the case in which one of the three adjacencies

\[ (i,j), (j,k), (i,k) \]

is not fulfilled the triplet is broken down to the pair that has the larger overlap according to Eq. 1. Note that removing or adding links can lead to the formation of new nontransitive triplets. However, because the triplets are composed of strictly increasing node numbers in the adjacency table, the correction is accomplished in a single pass through the data.

Figure A1D and E illustrate the preceding method using the data set that includes the data illustrated in Fig. A1B. Figure A1D presents the adjacency of the EMG pairs shown in Fig. A1C in graph-theory format. In these four muscles, no triplets can be formed because each burst overlaps only with its closest neighbor. In this case, the link between the PrT(1) and the Br is maintained, because these bursts show the greatest overlap, and the links between the Br and the BrR and between the TrM(1) and the PrT are removed. These results in the production of three clusters. However, both Br and TrM are free to form clusters with other muscles (as illustrated in Fig. 5). Figure A1E shows these same relationships as they appear in the adjacency table before (left) and after (right) the corrections for nontransitive neigh-
In other situations, a burst that does not initially form a triplet with any other pair may be added to a cluster in the final adjacency table. This is the situation for the Tri, which does not form a pair with the SSp. However, it does form a triplet with TriL and PaL and these latter two muscles form a triplet with SSp. A link between Tri and SSp is therefore added to the adjacency table (the green square in the table of Fig. A1D and the green dashed line in Fig. A1E).
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