Natural Neural Output That Produces Highly Variable Locomotory Movements

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Hooper, Scott L., Christoph Guschlbauer, Géraldine von Uckermann, and Ansgar Büschges. Natural neural output that produces highly variable locomotory movements. J Neurophysiol 96: 2072–2088, 2006. First published June 14, 2006; doi:10.1152/jn.00366.2006. We recorded fast extensor tibiae motor neuron activity during single-legged treadmill walking in the stick insect, Carausius morosus. We used this activity to stimulate the extensor muscle motor nerve, observed the resulting extensor muscle contractions under isotonic conditions, and quantified these contractions with a variety of measures. Extensor contractions induced in this manner were highly variable, with contraction measures having SDs of 12 to 51%, and ranges of 82 to 275%, when expressed as percentages of the means, an unexpectedly wide range for a locomotory pattern. Searches for correlations among the contraction measures showed that, in general, this high variability is not reduced by contraction measure covariation. Comparing responses (to identical input) across animals showed that extensor muscles from different animals generally significantly differed from one another. However, correlation analyses on these data suggested that these differences do not indicate that multiple extensor muscle subtypes exist. Extensor muscles instead appear to belong to a single class, albeit one with high animal to animal variability. These data thus provide another well-quantified example (along with Aplysia feeding) of a repetitive but highly variable motor pattern (in contrast to the high rhythmicity and stereotypy present in most other well-quantified repetitive motor patterns). We suggest this high variability could be an adaptive combination of locomotion, active sensing, and crypsis arising from the relatively low demand for locomotion in Carausius behavior, the highly fragmented environment the animal inhabits, and its need to avoid predatory attention.

INTRODUCTION

Understanding the neural basis of motor behavior is a fundamental goal of neuroscience. Repetitive motor patterns (such as breathing), in which a unit movement sequence (such as single breaths) are repeatedly produced, constitute a large proportion of motor behaviors. Numerous preparations have been developed in which the neural networks generating repetitive behaviors can be studied, including crustacean gill ventilation, swimmeret beating, and internal chewing and food filtering; Clione, lamprey, leech, and tadpole swimming; Aplysia feeding; leech heartbeat; locust flight; turtle scratching; and stick insect walking (Arshavsky et al. 1986; Brodfuehrer et al. 1995; Büschges 2005; Calabrese et al. 1995; Grillner and Wallén 2002; Hooper and DiCaprio 2004; Marder and Bucher 2001; Marder and Calabrese 1996; Mulloney et al. 1993; Robertson and Pearson 1985; Stein 2005; Tunstall and Sillar 1993; Weiss et al. 1992). The focus of much of this work was identifying the mechanisms by which nervous systems produce highly stereotyped and rhythmic outputs. Most of it was therefore likely conducted under conditions chosen to maximize system stereotypy and rhythmicity, and stereotypy and rhythmicity certainly constitute the primary focus of most of this literature.

This may have led to the belief that repetitive motor patterns are in general highly stereotyped and rhythmic. However, in Aplysia feeding irregular interbite intervals and bite amplitudes are the norm, and this high variability is an inherent property of the feeding neural network (Brezina et al. 2005; Horn et al. 2004; Zhurov et al. 2005). Human breathing, chewing, and copulation are also often produced with large variations in cycle period and the amplitude and phasing of the movements composing the repeating unit (Buschang et al. 2000; Plesh et al. 1987, 1988; Wintergerst et al. 2004). How common highly rhythmic stereotypy is and what functional advantages lead to stereotypy in some cases and high variability in others is thus also unclear. However, to better understand repeating motor behaviors in general, and to interpret properly work in this field, these are clearly important issues to investigate.

Another difficulty with much of the above work (again with the exception of Aplysia feeding) is that it examined system activity primarily on the neuronal level. However, muscles respond to motor neuron input in highly nonlinear manners. Moreover, because neural and muscular systems coevolved, many aspects of motor neuron activity are presumably tuned to the particular properties of the muscle the neuron innervates. Predicting movement from motor neuron spiking activity or relating motor neural network synaptic and cellular properties to behavior is thus impossible without knowing how muscles respond to input.

To help address these issues, we have quantified the response of Carausius morosus extensor muscles to motor neuron activity recorded from single-legged animals walking on a treadmill. We report here that, even under these highly constrained and unvarying conditions, extensor muscle contractions show great variability.

METHODS

All experiments were performed at room temperature on adult female Carausius from a colony maintained at the University of Cologne. All animals were approximately the same size and only animals that appeared to be in good health (as assessed by having robust responses to handling) were used.

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Neural recordings during treadmill walking

This technique was previously described extensively elsewhere (Gabriel et al. 2003). In brief, all legs except the right middle were amputated at midcoxa and the animal attached dorsal side up to a foam platform. Pro- and retraction of the remaining leg was prevented with dental cement (Protemp II, ESPE) and platform height adjusted so that at midstance femur–tibia and tibia–treadwheel angles of the right middle leg were 90°. Gut, fat, and connective tissue were then removed from the thorax. Throughout the experiment the thorax was filled with C. morosus saline (NaCl 178.54 mM, HEPES 10 mM, CaCl₂·2H₂O 7.51 mM, KCl 17.61 mM, MgCl₂·6H₂O 25 mM, pH 7.2). Nerve n13 (Marquardt 1940) activity was recorded with a monopolar hook electrode, digitized with a Micro1401, and stored on a personal computer using Spike2 software (both from Cambridge Electronic Design). Walking was induced by tactile stimulation of the abdomen or antennae. Six walking sequences (referred to as walking sequences A–F) from five animals were used in this work.

Muscle stimulation and recording

The preparation was the same as in the treadmill experiments except all femur movement was prevented with dental cement, the tibia was fixed to the side of the platform with an insect pin, and the extensor muscle tendon severed below the femur–tibia joint and attached to an ASI dual-mode lever system (Aurora Scientific). The ASI is a force and movement transducer in which the experimenter sets the maximum force the ASI will deliver to the muscle. On muscle contraction the ASI delivers sufficient force to prevent muscle shortening (standard isometric recording) until the set maximum force is reached, at which point the ASI delivers only the maximum force and the muscle therefore shortens against this constant load (standard isometric recording). Maximum force was always set at the minimum value necessary for the muscles to fully relax between all contractions (in one muscle the maximum force level was set too low, and four contractions that did not fully relax were thus not analyzed), and varied from 3.1 to 15.6 mN (mean 11.5 ± 9.4).

The Spike2 software was used to identify fast extensor tibiae (FETi) action potentials in the stored nerve recordings and to deliver for each FETi spike a 1-ms stimulation to n13 through a Model MH401 (designed by the Elektroniklabor Tierphysiologie at the University of Cologne) stimulus isolation unit and a bipolar stainless steel electrode isolated from the bath with white Vaseline (Engelhard Arzneimittel, Niederdorfelden, Germany). Current pulse amplitude was set slightly (10–20%) above contraction threshold, a level at which likely only the FETi motor axon was stimulated (Bässler and Storrer 1980). It is nonetheless possible that in some experiments the slow extensor tibiae (SETi) or common inhibitor (CI) axons, which have higher thresholds than that of FETi, were stimulated. However, under the experimental conditions used here, stimulation of these two axons in addition to FETi appears to have little or no effect on muscle contraction (Guschlbauer, personal communication). Furthermore, all muscles, when identically stimulated, showed very similar responses (see Fig. 1D, below). This observation suggests either that in all experiments the same set of axons were stimulated, or if different sets (in some experiments, FETi alone, in others, FETi plus SETi and/or CI) were being stimulated in different experiments, this variation had little effect on muscle response. Nerve stimulations and muscle contractions were simultaneously recorded with Spike2. Nerve n13 was generally crushed between the electrode and the ganglion with a forceps. Seven muscles, each from a different animal, and all from different animals from which the nerve recordings were obtained, were used. These experiments are referred to as muscle experiments (or sometimes just experiments) 1–7. Each muscle was driven by all six walking sequences recorded earlier from the five other animals (see above).

Contracting muscles have high metabolic needs, and “rundown” (e.g., muscle responsiveness declining over time) is consequently a considerable concern when working with muscles. Comparison of recordings to identical motor neuron stimulations showed that declining responsiveness did occur in extensor muscles (Fig. 1A; in this and all panels of this figure, the stimulations were the natural walking sequences obtained as explained above in Neural recordings during treadmill walking and the sequence letters refer to those used in Fig. 2). This figure shows five contractions of walking sequence B with the second, third, and fourth repetitions of the sequence being performed 36, 44, and 51 min after the first. The largest contractions were the muscle’s response to the first stimulation of the sequence and the progressively smaller responses, the responses to the later stimulations.

Although this figure shows that “rundown” occurs, it is a worst-case scenario in that a long time separated the first and the second stimulations, and the second, third, and fourth stimulations were done late in the experiment, in which “rundown” becomes pronounced. Figure 1B shows eight contractions from walking sequence A stimulated 14 min apart, but within the first 20 min of the experiment, and Fig. 1C shows four repetitions of 12 bursts from walking sequence E, taken over a 4-min period, again early in the experiment. In both cases the muscle contractions overlay extremely well (in Fig. 1C the overlay is so good that the four repetitions are indistinguishable). In five muscle experiments the walking sequence stimulations were performed first in the experiment and stimulation with all six walking sequences was accomplished within at most 20 min of start of the experiment (in four of the five, in <11 min); it is doubtful that significant rundown occurred during this time. In two experiments the walking sequence stimulations were performed 23 and 26 min after start of the experiment, respectively, but in these cases tonic nerve stimulations were performed before and after the walking sequence stimulations. In these experiments 1.7 and 3.6% rundown occurred over the period in which the walking sequence stimulations were performed, changes too small to account for the large variability in muscle response shown here (see Results).

The work presented here shows that the contractions induced by the neural activity observed during single middle legged walking are highly variable. The neural activity shows considerable step-to-step variability (data not shown), and a natural presumption is this variability in neural activity is the sole, or the primary, source of the contraction variability. However, another possibility is that the neural activity only triggers the contractions, the subsequent evolution of the contractions is otherwise independent of the neural firing pattern in the burst, and the muscles have endogenous properties such that they produce highly variably contractions, even when driven by very similar, or identical, neural inputs. Figure 1A–C shows this is not the case in individual muscles, in that the same patterns of contraction variation occur when a sequence is repeated. For instance, even in Fig. 1A, in which significant rundown occurs, in each repetition the first contraction is the largest, the second and third are second largest and of nearly the same amplitude, the fourth is slightly smaller, and the fifth is the smallest. Figure 1D shows the responses of all seven muscles to walking sequence D. Although there are some differences among the muscles, the burst-to-burst variations in contraction amplitude and shape are very similar in all muscles (this was true for all walking sequences; data not shown). Thus both in individual muscles and across muscles similar patterns of contraction variation are produced when neural patterns are repeatedly played, and thus much if not most (muscle specific variability is dealt with in Results) of the contraction variation shown here is attributed to changes in neural input.

Subsequent analysis

Spike2 nerve stimulation and muscle contraction data were transferred to Kaleidograph (Synergy Software) and analyzed using Kalei- dagraph built-in functions or macros written by the authors. Plots were
Burst identification

The bottom traces in the panels in Fig. 2 show nl3 stimulation exactly mimicking FETi motor neuron spiking activity in six walking sequences. The top traces in each panel show the resulting extensor muscle shortenings. The walking motor pattern was repetitive but highly variable. This variability raised two difficulties in defining neuron bursts. The first arises from the presence in these traces of some very long quiescent periods (the double-headed arrows in Fig. 2A mark particularly notable examples). These are a problem because sensory input induces a bout of stepping that then dies out. Unfortunately, when the experimenter stimulated the animal was not noted in our data. Thus when stepping resumed after the long quiescent periods in Fig. 2, it is unclear whether this occurred spontaneously (in which case the quiescence duration was a valid interburst interval) or instead was a result of the experimenter reinducing walking by tactile stimulation. To overcome this difficulty we noted that during robust walking (e.g., Fig. 2, C, E, and F) the step cycle period was about 1 s. It is therefore unlikely an experimenter would have believed that a walking bout had ended, and thus would have restimulated the animal, within 2 s after any step. To ensure that only interspike intervals (ISIs) belonging to stepping bouts were included in our data, ISIs > 2 s were therefore excluded from analysis.

The second difficulty is the highly variable spiking activity that occurs even within what is clearly a single stepping bout. Consider the bouts in Fig. 2A in the rectangles marked “1” and “2.” The first and second bouts consisted of five and six discrete muscle shortenings, respectively, which might lead one to believe that muscle shortenings could be used to define neuron bursts. However, this choice is invalid because muscle shortening depends on the ASI maximum force chosen by the experimenter. For instance, at the chosen ASI level several motor neuron “bursts” did not cause muscle shortening (asterisks). However, if the ASI level had been set low enough for these “bursts” to induce shortening, many shortenings that are presently discretely separate would have temporally summed into extremely long fused contractions. The associated stimulus trace shows that, although the spiking activity inducing the muscle shortenings was, in general, arranged into perceptually apparent bursts, these “bursts” had a wide range of interburst intervals and durations. It was thus unclear what ISI should be used to define minimum interburst interval.

These considerations led us to define interburst interval as follows. In a perfectly rhythmic system in which the ISI inside bursts is $x$ ms and the ISI between bursts is $y$ ms, a histogram of ISI will have nonzero values only in bins $x$ and $y$. As the system becomes less perfectly rhythmic a range of ISIs centered around $x$ will occur inside

FIG. 1. Nerve stimulations performed within relatively short intervals produce similar contractions within single muscles and across muscles. Muscle “rundown” occurs if nerve stimulations are performed far apart, particularly late in the experiment (A), but does not when stimulations are performed within shorter durations (B and C). Different muscles show similar contraction patterns when identically stimulated (D). See text for further explanation.
the bursts and a range of ISIs centered around y will occur between the bursts and the histogram will become a two-peaked distribution with maxima at x and y and a minimum between them. As the system becomes even less rhythmic the depth of the minima will decrease, with the distribution becoming flat when bursting activity is replaced by simple tonic firing with variation. To decide which ISI should be the minimum interburst interval, we therefore constructed a histogram of all ISIs in our data set (Fig. 3). This figure showed a minimum in the 0.2- to 0.24-s bin. An ISI of 0.2 s was therefore chosen as the minimum interval separating two FETi bursts. Comparison of the bursts defined in this manner with the muscle shortenings showed good agreement (i.e., in no case did what appeared to be single muscle contractions contain two neuron bursts, nor did single neuron bursts ever give rise to what appeared to be multiple muscle contractions).

Geometrical relationships among certain contraction measures

Among other measures, we quantified contraction maximum amplitude, rise slope, rise duration, fall slope, and fall duration. Maximum amplitude, rise slope, and rise duration and maximum amplitude, fall slope, and fall duration have geometrical relationships that are important in interpreting certain of the data presented here. Consider first maximum amplitude, rise slope, and rise duration. In real contractions maximum amplitude approximately equals rise duration times rise slope (see Fig. 6 in RESULTS). Figure 4 shows schematic contractions in which this relationship is exactly true. Maximum amplitude can therefore be increased by increasing rise duration alone (Fig. 4B), increasing rise slope alone (Fig. 4C), increasing both (Fig. 4D), or decreasing one but increasing the other enough that their product still increases (Fig. 4E; in this example slope decreased and duration increased). Identical considerations exist for fall slope and fall duration, given that maximum amplitude also equals fall slope times fall duration. Thus although in Fig. 4 fall slope was kept constant, the increased amplitude in Fig. 4, B–E could have been associated with any combination of changes in fall slope and duration provided their product equaled the new amplitude.

Statistical comparisons (Fig. 12, Tables 1 and 2)

The linear relationship equations in Table 1 were obtained as follows: For each muscle, which contraction measures (Fig. 6) significantly and consistently correlated were identified as explained in RESULTS describing Fig. 9. For measures with a significant and consistent correlation, the linear fit equations of the significantly correlated walking sequences were averaged to obtain the average linear fit between the two measures for that muscle. This procedure resulted in, for each muscle, a matrix of average linear fits for each significantly and consistently correlated contraction measure pair. The matrices of the seven muscles were then compared to identify which correlations were present in a majority of the muscles. For correlations for which this was true, whether the slopes of the average linear fits of the muscles differed from zero was then tested using a Student’s t-test. If this was also true, the average fit equations of the individual muscles were then averaged to give the equations in Table 1.

For example, two measures used to define the contractions (see Fig. 6), rise duration and 0.05-mm duration, were significantly correlated in muscle 6 (Fig. 10C) in five walking sequences, and the slopes of linear fits to these five walking sequences differed from zero by a Student’s t-test. The linear fit equations of these five walking se-
The slopes of the four individual muscle equations (muscle 2, 0.43; muscle 4, 0.35; muscle 6, 0.49; muscle 7, 0.31) differed from muscles. The slopes of the four individual muscle equations (muscle and so analogous average equations were calculated for these three 0.05-mm duration were also consistently and significantly correlated, 0.05-mm duration. In three of the other six muscles rise duration and amplitude increased as the result of an increase in rise duration, in \( t \) to be performed, resulting in an adjusted \( \alpha \)-level of 0.07. However, because four contraction measure pairs existed in which the majority of the muscles individually showed a correlation, a Dunn–Sidak compensation for four simultaneous comparisons had to be performed, resulting in an adjusted \( \alpha \)-level of 0.013. In the case at hand, the \( t \)-test probability (0.002) met this significance criterion and the equations of the four muscles were therefore averaged to obtain Eq. 4 in Table 1.

A concern with these analyses is their use of Student’s \( t \)-test because there were only six walking sequences and seven muscles, which are too few points for meaningful normality tests. It is thus unclear whether these \( t \)-tests are valid. Unfortunately, these numbers of points are also too few (due to the Dunn–Sidak compensation required for multiple comparisons) for the Wilcoxon sign/rank test to be significant even if all walking sequence equation slopes, or all individual muscle equation slopes, had the same sign. If it is incorrect to use \( t \)-tests on these data, this would mean that we are unable to assess significance in these data. However, this in no way affects the main point presented here, that extensor muscle contractions are highly variable. It would simply mean that the observed correlations, which at present reduce this variability by linking together different contraction measures, could no longer be accepted.

This concern is not present in the analyses in Fig. 12 and Table 2 because in these cases sufficient data points (210 and 178, respectively) were available for Wilcoxon sign/rank tests to show significance. The seven muscles in Fig. 12 were identically stimulated and thus a paired Wilcoxon sign/rank test was used. The equations in Table 2 were obtained in a manner exactly analogous to that for Table 1. In brief, for each contraction measure pair, the data in each of 178 plots similar to Fig. 14, B1–B3 were tested for correlation; to be a consistent correlation over half the plots had to show a significant correlation. For correlations meeting this criterion, the slopes of linear fits to the data in the plots with significant correlations were tested for difference from zero using an unpaired Wilcoxon sign/rank test. For each correlation in Table 2 this test gave values of \( P < 0.0001 \). The linear fit equations of the plots with significant correlations were then averaged to obtain the equations in Table 2.

RESULTS

Here we present data showing that extensor muscle contractions driven by neural sequences recorded during single middle leg walking are highly variable. These data were obtained by first recording neural activity in six (A–F) walking sequences and then using these recorded sequences to drive extensor muscle contractions in seven other animals (experiments 1–7). In each of these animals all six walking sequences were used. We first show the data from one animal and then compare the responses of all seven animals.

Response of a single extensor muscle to nerve stimulations mimicking walking

Figure 2 shows that, when one extensor muscle is induced to shorten (top traces) by motor nerve stimulation (bottom traces) exactly mimicking the fast extensor tibiae (FETi) motor neuron spiking present in six walking sequences (A–F), a wide variety of contraction amplitudes and durations, and of intercontraction intervals, are present. To perform more detailed analyses we divided the spike trains into bursts (see METHODS) and examined the contractions each burst induced; Fig. 5 shows eight examples.

Two points about this figure are important. First, when watching treadmill walking many observers perceive two movement types; two contraction types appear to be present in our muscle data. The first movement type is stepping, which almost certainly corresponds to the relatively short and simple contractions in Fig. 5, A–G (Fischer et al. 2000; Gabriel and Büschges 2006). The second movement type is searching, in which the animal performs multiple extensions and flexions while keeping the leg elevated and which may correspond to very long duration contractions with multiple lengthening–shortening cycles such as that in Fig. 5H (Bässler 1993; Gabriel and Büschges 2006). To test whether two contraction types exist, we sorted the contractions by eye according to whether they appeared to be steps or searches, primarily on the basis of whether multiple lengthening–shortening cycles were present. We then separately analyzed (see following text) the two classes to test whether they were statistically separate and, if so, by which measures. The second point is the variability present even in contractions almost certainly corresponding to steps (Fig. 5, A–G). At least seven characteristics vary: amplitude (A, B, C, and G vs. D, E, and F), duration (B and C vs. all others), rise rate (D vs. A, C, E, and G), relaxation rate (E vs. all others), whether the rises or relaxations contain multiple slopes (F and G), and whether the contractions more closely resemble a triangle (A) or a trapezoid (C).
To quantify these variations we measured (Fig. 6) intercontraction interval and contraction cycle period, maximum amplitude, duration at amplitudes of 0.05 mm and 50 and 90% maximum amplitude, rise and fall slopes (the slopes of linear fits to the data between 0.05 mm and 90% maximum amplitude), and durations (again, defined relative to 0.05 mm and 90% maximum amplitude). We also calculated two measures related to how triangular or trapezoidal the contractions are. The first is 90% maximum amplitude duration divided by 0.05-mm-amplitude duration, which should decrease as contractions become more triangular. The second (expressed as a percentage) is maximum amplitude divided by the height of the triangle formed by the rise and fall fit lines, which should increase as contractions become more triangular (although both measures are more difficult to interpret for contractions with complex shapes such as those in Fig. 5, F and G).

Figure 7 shows cycle period, intercontraction interval, maximum amplitude, and duration data in one muscle for all six walking sequences in Fig. 2 (six leftmost data columns in each panel). Black data points are from contractions identified as likely steps and red points from likely searches. The two rightmost data columns in each panel are the mean, SD, and range (triangles) of the data from all contractions (column 7) and with the data from likely searching movements excluded (column 8). The traces below each panel are the contractions with the minimum (red), 1/3 maximum (purple), 2/3 maximum (green), and maximum (blue) value of the contraction measure in question using data only from likely steps (only the minimum and maximum are shown for cycle period). Figure 8 shows rise and fall slope, rise and fall duration, percentage triangle height, and 90% amplitude duration/0.05-mm-amplitude duration using the same conventions, except that for rise and fall slope a contraction with a 1/6 maximum value of the measure in question is also shown (yellow).

Two points about these data are important. First, although the black (likely steps) and the red (likely searches) data often differ (Fig. 7, A, D, and E; Fig. 8, B, D–F), these contraction measures generally do not unambiguously separate the groups. For instance, in the walking sequences (A, D, and F) in which likely searches occurred, 0.05-mm and 50% maximum amplitude duration tended to be longer in likely searches than in likely steps (Fig. 7, D and E). However, in each case the durations of over half the likely searches were less than the largest corresponding duration of walking sequence C, which had only likely steps. Fall slope (Fig. 8 B) best separated the two classes; when –0.5 is used as a boundary value only two (out of 15) likely searches are classified as steps and only one (out of 178) likely step as a search.

Second, even when likely searches are excluded, the contraction measures show very large variability, with coefficients of variation (SD/mean) ranging from 12% (percentage triangle height) to 51% (90% amplitude duration) and ranges (as a percentage of the mean) from 82% (percentage triangle height) to 275% (rise duration). Furthermore, in most cases (fall duration being an exception in that two data points are responsible for roughly one third of the range) the data are fairly well

**Fig. 5.** Representative contractions (from those in Fig. 2) demonstrating the wide variety of contractions induced by motor nerve stimulation mimicking motor neuron firing during single-legged treadmill walking.
Correlations between contraction measures only moderately reduce movement variability

If the measures in Figs. 7 and 8 are independently assorted (e.g., contractions of a given maximum amplitude can have any rise duration), these data define a 12-dimensional space in any region of which contractions can exist. Alternatively, if some contraction measures are correlated (for instance, large maximum amplitudes always have long rise durations and small ones short rise durations), then one measure fully describes the other, only one of the two measures need be determined, and the space of possible contractions is reduced by an entire dimension. We therefore tested for correlation of intercontraction interval and of 0.05-mm-amplitude duration with cycle period, and of all possible pairings (45) among maximum amplitude; 0.05-mm, 50% maximum, and 90% maximum amplitude durations; rise and fall slopes; rise and fall durations; percentage triangle height; and 90% duration/0.05-mm duration.

Figure 9A plots intercontraction interval against cycle period for each walking sequence (color code, A–F). In each sequence intercontraction interval and cycle period were correlated at the 0.05 level (asterisks), but the correlation was not as good for sequence C, and for sequences C and E the dependency had a slope of about 0.5, whereas for the others the slope was about 1.0. Cases in which two measures were significantly correlated in only some walking sequences, or in which the pair was significantly correlated in all sequences but the dependencies had widely varying slopes, often occurred in these analyses.

To deal with this variability we performed the following two-step analysis. We first tested each contraction measure pair for correlation (nominal α-level, 0.05) individually for each walking sequence. We then defined significant and consistent...
correlations as those in which three or more of the walking sequences had significant correlations, and a Student’s t-test on the slopes of linear fits to the walking sequences with significant correlations showed that this set of slopes differed from zero. By this standard, intercontraction interval correlated with cycle period because the measures were significantly correlated in all six walking sequences and the t-test value of the slopes of fits to the data from these six sequences was 0.00029 (because we performed 47 comparisons, a multiple comparison Dunn–Šidák compensated α-value of 0.0011 was used). Figure 9B shows 0.05-mm duration plotted against cycle period. These contraction measures were significantly correlated in walking sequences C and E only, and thus by our criteria 0.05-mm duration did not consistently depend on cycle period.

Figure 10 shows consistent significant correlations between 50% maximum amplitude duration and 0.05-mm duration (Fig. 10A), 90% maximum amplitude duration/0.05-mm duration and percentage triangle height (Fig. 10B), and rise duration and 0.05-mm duration (Fig. 10C). These correlations are not unexpected. For triangular or trapezoidal contractions, durations at different amplitudes should be correlated from geometry. Ninety percent maximum amplitude duration/0.05-mm duration and percentage triangle height both quantify contraction shape, and thus again could well be correlated. For 0.05-mm duration to increase, one, some, or all of rise duration, fall duration, or 90% maximum amplitude duration must increase; Fig. 10C shows that for this extensor muscle, rise duration consistently increased. Consistent significant correlations also existed between 90 and 50% maximum amplitude durations, rise slope and 50% maximum amplitude duration, rise duration and 50% maximum amplitude duration, rise duration and rise slope, and fall duration and percentage triangle height. With the exception of the correlation between 90 and 50% maximum amplitude durations (which is again expected on geometrical grounds), these other correlations were not ob-
served in all muscles (see following text) and we shall not consider them further here.

What is notable is not the presence of these other correlations, but rather the lack of correlations that might be expected. For perfectly linear contractions, maximum amplitude equals rise slope times rise duration (see METHODS). This relationship shows that maximum amplitude can be changed by changing either rise slope or rise duration alone, or both together. Despite these multiple ways in which maximum amplitude can increase, vertebrate skeletal muscle contraction amplitude typically increases as a result of increased motor neuron spike frequency or recruiting additional motor units, both of which increase primarily rise slope. Extensor muscles could similarly generally change contraction amplitude in only one way, for instance, by increasing rise slope but not rise duration. Identical considerations apply to fall slope and fall duration, given that maximum amplitude also equals fall slope times fall duration. For the falling phase of the contraction, one might expect changes in maximum amplitude to arise primarily from changes in fall slope. This arises because in most situations contraction relaxation occurs after motor neuron firing has ceased and thus depends only on muscle intrinsic properties. Many muscles relax exponentially and the slope of an exponential decay increases as its initial amplitude increases. This property means that, if linear fits are performed on the contraction relaxations (as was done here; Fig. 6), one expects the fall slope to increase more rapidly than fall duration as contraction amplitude increases.

The extensor muscle data fulfill none of these expectations. With respect to rise duration (Fig. 11A), although in four experiments a significant correlation existed, considerable scatter is present. The slopes of the dependencies (rise duration increased with maximum amplitude in three of the four significant experiments but decreased in the fourth) showed sufficient variation that no consistent correlation existed. Similar large scatters and inconsistent slopes were also present in the rise slope, fall duration, and fall slope plots (Fig. 11, B, D, and E). To ensure that an analysis error was not occurring, we also plotted rise slope times rise duration and fall slope times fall duration against maximum amplitude (Fig. 10, C and F); as they must, the products had small scatters and consistently and significantly correlated with maximum amplitude. These data thus indicate that rise duration and rise slope, and fall duration and fall slope, can change independently as extensor muscle maximum amplitude changes.

![Figure 9](image_url)  
**FIG. 9.** A contraction measure pair (intercontraction interval and period) that was consistently and significantly correlated (A) and one (0.05-mm-amplitude duration and period) that was not (B). Color coding refers to the 6 walking sequences in Fig. 2. Asterisks indicate significant correlations.

![Figure 10](image_url)  
**FIG. 10.** Three additional contraction measure pairs that significantly and consistently correlated. Figure conventions are identical to those in Fig. 9.
Responses of extensor muscles from other individuals

The data presented thus far are from an extensor muscle from one animal. To determine whether these data were representative of extensor muscles in general, we stimulated six more extensor muscles with the walking sequences shown in Fig. 2. We first repeated on these muscles the measurements shown in Figs. 6–8. For simplicity we do not present here each muscle’s data sorted by walking sequence (columns 1–6 in Figs. 7 and 8), but instead show only the means, SDs, and ranges of the grouped data excluding likely searching movements (the equivalent of column 8 in Figs. 7 and 8) (Fig. 12). The muscle shown in Figs. 7 and 8 is muscle 6 in Fig. 12. The horizontal lines beneath each panel indicate how the muscles differ from one another (see figure legend).

Three points are important about these data. First, all muscles showed large SDs and ranges for all contraction measures and thus the large variability shown earlier for a single extensor muscle was present in all extensor muscles examined. Second, there appeared to be two contraction measure classes, one with large muscle-to-muscle variation (maximum amplitude, rise and fall slope, fall duration) and one with small muscle-to-muscle variation (0.05-mm duration, 50 and 90% maximum amplitude duration, rise duration, percentage triangle height, 90% maximum amplitude duration/0.05-mm duration). Averaging each measure’s individual muscle means and calculating the coefficient of variation of these across-muscle means showed that this perception was real, with maximum amplitude, rise and fall slope, and fall duration having coefficients of variation (CVs) >0.22 and the other measures having CV values <0.14 (Fig. 12K), and the two groups forming a distribution with two well-separated peaks (data not shown).

Third, despite being identically stimulated, different muscles generally had statistically different responses [ranging from a low of 13 of the 21 comparisons (62%) differing for 90% maximum amplitude duration to a high of 19/21 (90%), differing for rise slope and fall duration]. To understand the basis of these differences, we examined the maximum amplitude responses of the seven muscles in detail. Figure

![Graphs showing statistical analysis](image-url)
13A shows the contractions induced in two of the muscles by ten motor neuron bursts. The maximum amplitudes of both muscles change in synchrony as the burst spike pattern changes, although the amplitude of the muscle shown in black is always larger than that of the muscle shown in red. Consequently, although the maximum amplitudes of the two muscles across these ten bursts significantly differ in a Student’s paired t-test, their activity is nonetheless very highly correlated.

Figure 13B shows the contractions of the muscle shown in black in Fig. 13A and of a third muscle (red) in response to the same ten bursts. The maximum amplitude of the muscle shown in black changes much more when neural input changes than does that of the muscle shown in red. That is, the first three bursts induce large contractions in both muscles, but the contractions shown in black are larger than those shown in red. Alternatively, when the bursts induce small contractions (bursts 6 and 7), the contractions shown in black are smaller than those shown in red. This shifting in which muscle produces larger contractions results in these two muscles not significantly differing in a paired t-test. However, in both muscles the sign of the changes in maximum amplitude that occur as the neuron bursts change is nonetheless the same for all bursts and there is no long-term shift in either muscle’s average contraction amplitude. The activity of the two muscles as neural input changes is therefore again highly correlated.

Figure 13CI shows the contractions of the muscle shown in black in Fig. 13, A and B, and of a fourth muscle (red), in response to a different set of 24 bursts. In this case the average activity of the two muscles diverges, in that the maximum amplitudes of both decline from contractions 3 to 7, but then the amplitude of the muscle shown in black increases to contraction 20, whereas the amplitude of the muscle shown in red maintains (with some contraction to contraction variation) a relatively constant amplitude over the same time period. These long-term changes result in the maximum amplitude of the muscle shown in red being larger than that of the muscle shown in black in contractions 1 to 9, but being smaller in contractions 9 to 24. These differences are insufficient to be significant in a paired t-test comparison but are large enough that the activity of the two muscles is uncorrelated.

FIG. 12. Summaries of contraction measures (A–J) in different muscles (columns 1–7 in each panel) driven by the walking sequences in Fig. 2, and the coefficient of variation of each contraction measure’s overall mean (K). All muscles show large variation in all measures. Each column shows mean, SD (error bars), and range (triangles). Horizontal lines below each plot indicate whether the muscles differ from one another using the following convention: the top line compares muscle 1 to all other muscles, with ✖ showing significant difference and ○ no significant difference. Second line compares muscle 2 to muscles 3 and greater, the third muscle 3 to muscles 4 and greater, and so forth. Because all muscles were stimulated identically, paired Wilcoxon sign/rank tests were performed with a nominal 0.05 α-level (Dunn–Sˇida´k compensated value for 210 comparisons, 0.00024).
However, this lack of correlation is misleading because, although their magnitudes are sufficiently different to give rise over many contractions to the observed long-term shifts, the signs of the amplitude changes from burst to burst are very similar. For instance, from contractions 7 to 15, over which most of the long-term increase of the maximum amplitude of the muscle shown in black occurs, both muscles show the same pattern of amplitude changes: increase, decrease, increase, decrease, increase, decrease, decrease, increase. One method to quantify this burst-to-burst similarity is to subtract the maximum amplitude of the preceding contraction from each contraction’s maximum amplitude. This procedure gives only the relative change in amplitude from one burst to the next and thus suppresses long-term shifts. When these data are plotted for both muscles (Fig. 13C2), they show the burst-to-burst changes of the two muscles are almost always in the same direction (in only three cases do they differ, indicated by asterisks) and that the activities of the two muscles are highly correlated.

When similar analyses were performed on the other contraction measures, situations analogous to those shown in Fig. 13, A–C were observed. This work thus indicates that the significant differences shown in Fig. 12 can arise either from the activities of two muscles being persistently displaced from one another (as in Fig. 13A) or because the activities of the two muscles have different long-term shifts throughout the experiment (similar to but more pronounced than that in Fig. 13C). However, in both these cases, and also in cases where the muscles did not significantly differ, the muscle activities were always highly correlated.

We also performed on the six new extensor muscles the same correlations between contraction measures that we had on the first. This analysis showed that many correlations were present in only a few muscles. For instance, only in the muscle shown in Figs. 7 and 8 were rise duration and slope, and fall duration and percentage triangle height, correlated, and this muscle was one of only two in which rise slope and 50% maximum amplitude duration, and rise duration and fall slope,
were correlated. We were again interested only in correlations that were present consistently and significantly across muscles. To assess this issue we followed a two-step process similar to that performed in the individual muscles (see METHODS). Only four correlations (out of 45 possible) were consistently and significantly present in a majority of the seven muscles. The geometries of triangles or trapezoids predict three of these (50% maximum amplitude and 0.05-mm durations, 90 and 50% maximum amplitude durations, 90% maximum amplitude duration/0.05-mm duration, and percentage triangle height). The fourth correlation was between rise duration and 0.05-mm duration (see Table 1 for average correlation equations). This was the only correlation between geometrically free contraction measures, in that 0.05-mm duration could also have increased by fall duration or 90% maximum amplitude duration increasing (see Fig. 6).

Until now we have performed correlations on data in which contractions were altered by changing neural input. Having data from multiple muscles allows an orthogonal correlation analysis to be performed, in which contractions are altered by changing muscle identity while keeping neural input constant. Because this is the second correlation analysis being performed, it is important to clearly distinguish the two. Figure 14A shows schematic contractions of four muscles induced by three neuron bursts (fifth trace). Muscle A (row 1) produces different amplitude contractions in response to the three bursts. These changes are a result of changes in rise duration (note horizontal and vertical dashed lines), not in rise slope (the sloping dashed lines on the second and third contractions indicate the first contraction’s rise slope). Rise duration and maximum amplitude, but not rise slope and maximum amplitude, are thus correlated in muscle A. Muscles B, C, and D also produce different amplitude contractions in response to the three bursts (rows 2, 3, 4) and, like muscle A, these changes occur because rise duration changes as neural input changes. Thus when a correlation analysis is performed for each muscle as neural input is changed, it will show that in each muscle rise duration and amplitude are correlated (a “significant consistent” correlation). This “horizontal” analysis, in which 1) differing neural inputs are used to induce contraction variability, 2) correlation analyses are performed individually on each muscle, and 3) the muscles are compared with see whether they show similar correlations, is what has been performed until now.

However, when data on multiple muscles are available, a correlation can be performed in another dimension, down the columns in Fig. 14A. That is, in column 1, in which all the muscles received identical input, the muscles nonetheless produced different amplitude contractions (explained by, for in-

| TABLE 1. Average significant and consistent correlations obtained by varying neural input with muscle identity constant and testing for consistency across different muscles (rows in Fig. 14) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| 50% duration = (−0.04 ± 0.03) + (0.81 ± 0.06) × 0.05-mm duration | 7/7  |
| 90% duration = (−0.05 ± 0.01) + (0.66 ± 0.06) × 50% duration | 7/7  |
| 90% duration/0.05-mm duration = (0.97 ± 0.07) − (0.009 ± 0.0008) × % triangle height | 7/7  |
| Rise duration = (0.01 ± 0.03) + (0.40 ± 0.07) × 0.05-mm duration | 4/7  |

The ratios (a/7) indicate in how many of the seven muscles the correlation was observed. See METHODS and the text in RESULTS describing Fig. 9 for an explanation of how these equations were obtained.

FIG. 14. A: schematic demonstrating correlations across multiple neural patterns within single muscles (rows, equivalent to correlations in Figs. 9–11) and of correlations across multiple muscles for single neural patterns (columns). When amplitude changes because the neural pattern changes, rise duration and amplitude are correlated. When amplitude changes because muscle identity changes, rise slope and amplitude are correlated. B1–B3: correlation plots of rise slope vs. maximum amplitude for real data. B1: correlation of the 2 measures across the 7 muscles for the first (B1), second (B2), and third (B3) bursts in Fig. 2A. Numbers next to data points indicate which muscle gave rise to the point. In all cases a significant correlation existed.
Extensor muscle movement is highly variable

...stance, the muscles being differently loaded or in different metabolic states). Whether this variation in contraction amplitude is correlated with variation in some other contraction measure can now be tested. In all four contractions in column 1 rise duration is constant but rise slope changes (the dashed lines on contractions of muscles B, C, and D indicate muscle A’s rise slope). When the muscles are driven by the second and third neural patterns, the amplitude changes that occur as muscle identity changes are also the result of changes in rise slope. These two correlation analyses thus show that when a muscle produces different contraction amplitudes because the neural pattern driving it changes, the amplitude changes are due to rise duration changing, but when contraction amplitude changes because muscle identity changes, the amplitude changes are due to rise slope changing.

Turning now to the real data, the correlations in Figs. 9–11 all had six fits. These arose because six walking sequences, each with 10 to 50 neuron bursts (the data points in Figs. 9–11), were used to stimulate each muscle. Each plot thus has data from only one muscle. The analysis being performed now is across multiple muscles driven by single bursts. The associated plots therefore contain data from all seven muscles, one plot per burst. Figure 14B1 shows this analysis for rise slope versus maximum amplitude for the first burst in Fig. 2A. The data points are the rise slope and maximum amplitude of the contractions this burst induced in the seven extensor muscles (numbers next to points). Figure 14, B2 and B3 shows the same analysis for the contractions induced by bursts 2 and 3. Because there are 178 bursts in Fig. 2, a complete analysis of the correlation between rise slope and maximum amplitude as muscle identity changes consists of 178 plots similar to Fig. 14, B1–B3. Significance and consistency were assessed in this across-muscle analysis exactly as in the within-muscle analysis. That is, to be accepted a majority of the 178 correlations had to be individually significant and in this analysis the (see methods) Wilcoxon sign/rank test probability of the slopes of linear fits to the significant cases had to differ from zero.

Across-muscle correlations were performed on all (45) pairings of the measures shown in Fig. 6. Five consistent, significant correlations were found (Table 2). Geometry predicts three of these (50% maximum amplitude duration and 0.05-mm duration, 90 and 50% maximum amplitude durations, 90% maximum amplitude duration/0.05-mm duration, and percentage triangle height). Consistent, significant correlations also existed between two geometrically free pairs, rise slope and maximum amplitude and fall duration and 0.05-mm duration. These correlations suggest that, whatever it is that causes identical input to induce in different muscles contractions with different maximum amplitudes and 0.05-mm durations, these changes are achieved, at least in part, by changing rise slope and fall duration, respectively. The lack of a correlation between maximum amplitude and either fall duration or fall slope is again notable, given the geometrical requirement that one or both of these measures change when maximum amplitude changes. These absences of correlation presumably indicate that, similar to the analysis shown in Fig. 11 for a single muscle, which of, and how, fall slope and fall duration change as maximum amplitude changes varies in a muscle-specific fashion.

Discussion

We have shown here that stimulations mimicking natural motor neuron activity produce highly variable responses in single extensor muscles and that extensor muscles from different animals respond differently to identical input. We discuss the data in reverse order.

Extensor muscles from different animals are different—multiple extensor muscle types or variation about a mean?

Extensor muscle biomechanical properties were previously measured with the goal of building extensor muscle models for dynamic stimulations of insect walking and biology-based neural controllers (Ekeberg et al. 2004). In this (and similar work in other species) data were gathered with the implicit assumption that extensor muscles from different individuals, although not identical, would belong to a single class (i.e., form a single peaked distribution). However, our data showing that by many measures extensor muscles from different individuals statistically differ from one another (Fig. 12) could indicate that multiple extensor muscle subtypes exist or that the different muscles were in different hormonal or modulatory states. In either case averaging data across muscles would then be incorrect. However, the muscles all responded similarly to changes in neural input, at least on a burst-to-burst level (Fig. 13), consistent with extensor muscles constituting a single population, albeit with considerable animal-to-animal variation. The most parsimoniuous conclusion is thus that our data do not invalidate averaging extensor muscle data across animals.

Extensor muscles from different animals are different—why are some measures more variable than others?

Maximum amplitude, rise and fall slope, and fall duration had larger muscle-to-muscle variability than did the other contraction measures (Fig. 12). The small variability in the other measures likely arises because muscle loading was set at the minimum necessary to prevent intercontraction summation. This procedure tends to equalize 0.05-mm duration because this is the duration that, to prevent summation, must be shorter than the intercontraction interval. Given the geometric, algebraic, and correlation relationships between 0.05-mm duration, 50 and 90% maximum amplitude durations, 90% duration/0.05-mm duration, and percentage triangle height, that these measures vary little when 0.05-mm duration varies little is not surprising. Fall duration does show large variability. This

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**Table 2.** Average significant and consistent correlations obtained by varying muscle identity with neural input constant and testing for consistency across different neural inputs (columns in Fig. 14)

| 50% duration | 138/178 | (0.08 ± 0.29) + (0.52 ± 0.2) × 0.05-mm duration |
| 90% duration | 130/178 | (−0.15 ± 0.18) + (0.9 ± 0.39) × 50% duration |
| 90% duration/0.05-mm duration | 159/178 | (1.1 ± 0.21) − (0.01 ± 0.0003) × % triangle height |
| Rise slope | 169/178 | (0.12 ± 0.26) + (4.5 ± 0.26) × maximum amplitude |
| Fall duration | 104/178 | (−0.14 ± 0.07) + (0.53 ± 0.11) × 0.05-mm duration |

The ratios (s/178) indicate in how many of the 178 muscles the correlation was observed. See Methods for an explanation of how these equations were obtained.
presumably occurs because 1) what variation there is in 0.05-mm duration is expressed in fall duration more than the other durations and 2) fall duration has relatively small mean values (given that \( CV = SD/\text{mean} \)). Preventing intercontraction summation required that the muscles be differently loaded, these different loads presumably being the reason for the large variation of maximum amplitude. Highly variable maximum amplitude would be expected to give rise to highly variable rise and fall slopes because of the geometrical relationships between maximum amplitude and rise slope and duration and fall slope and duration (Fig. 4).

This analysis suggests that which contraction measures will show high variability depends on experimental conditions. That is, the muscles could have been loaded to equalize maximum amplitude. Because changing muscle load changes contraction duration, in this loading regime 0.05-mm duration (and presumably many of the other measures presently showing little variation) would likely be highly variable. However, because of the correlation between maximum amplitude and rise slope, in this case rise slope presumably would also have little variation. It would therefore be incorrect to conclude from the data in Fig. 12E that the different muscles have different inherent contraction velocities because a different loading paradigm would have likely equalized rise slope. The correct interpretation is instead that, although these data show the muscles are different, they do not reveal the fundamental nature of the difference because which measures express the variability will change depending on experimental conditions. Similar dependency of measure variability on experimental conditions, and an inability to directly associate which measure shows high variation with the variation’s fundamental nature, is likely a property of many complex systems.

**Extensor muscles from different animals are different—implications for neural control**

The variation in muscle properties between animals means that, to produce a given contraction, individual animals would need to “know” these differences and alter their motor neuron firing to compensate for them. *Carausius* are unlikely to be able to achieve this goal by using sensory feedback to correct for differences between “desired” and achieved contractions because of the slow response properties of the extensor muscles (unpublished observations). Alternatively, each animal’s walking network could be “trained” during development to produce motor neuron bursts appropriate to the muscle it innervates.

Another possibility is that the variation in muscle properties is simply not compensated for. This idea may seem contrary to function. In humans it is the equivalent, if both quadriceps were not equally strong, of being unable to increase neural input to the weaker muscle to maintain equal swing distances in both legs. However, the middle leg extensor muscle primarily serves to increase tarsus altitude above the substrate during swing and tarsus protraction distance at the end of swing. Provided these goals are met, fine control of leg extension may not be important. This is particularly true in the tested situation, single-leg treadmill walking, in which the substrate is at a fixed altitude (Bässler 1993; Gabriel and Büschges 2006). However, we also argue below that, under natural conditions, highly variable extensor muscle contractions could be advantageous.

If so, there may be even less need to compensate for interanimal variation. That is, if the goal with each step is to produce, in an essentially random fashion, a different extensor muscle contraction, then it is unclear it would ever be necessary that the animal “know,” and tailor its motor neuron output to, the specific properties of its own muscles.

**Extensor contraction during walking is highly variable**

When driven by extensor motor neuron firing recorded during single-legged walking, extensor muscle contractions are highly variable. This variability reflects highly variable motor neuron firing, but had the variability been measured on the neuron level, the extent to which the muscle expressed it would have been unknown. Measuring instead muscle response greatly increases the likelihood that the variability is behaviorally relevant. These findings extend the investigation of stick insect walking variability from gait selection (Cruse 1990; Graham 1985) and single-leg movements (Bässler 1993) to individual muscles. Correlations among contraction measures only moderately reduced the observed variability. Notable among these is a dependence of intercontraction interval on period. This result agrees with work showing that, in both single-leg walking (Gabriel and Büschges 2006) and intact insects walking with a tetrapod gait (Bässler 1983; Graham and Cruse 1981), only stance phase motor neuron activity depends on period.

**Extensor contraction during walking is highly variable—comparison with prior work**

Stick insect walking has been studied for >80 yr (von Buddenbrock 1921). Measurements taken from figures or calculations made from tables in these articles indicate that the high variability shown here for a single muscle during single middle leg treadmill walking is also present in other single-leg conditions, as well as under more intact and more natural conditions. In work examining single front leg movement, the measured parameter has a CV of 0.26, well within the range of CV values (0.15–0.51) of the measures taken here (Bässler 1993).

Protraction duration of intact animals walking on oil (Epstein and Graham 1983) and protraction and retraction duration and leg movement amplitude when walking on a treadmill (Wendler 1965) have CV values ranging from 0.13 to 0.4. Posterior extreme position (PEP, just before leg lifting at the end of stance) for middle and hind legs of stick insects walking on silicon oil have ranges roughly 70% of the mean, with the data smoothly spread across this range (e.g., the ranges are not attributed to a few outliers) (Cruse and Segev 1988). Protraction duration and period of intact animals walking on mercury can have ranges (as a percentage of the mean) as great as 150%, leg amplitude and step period under the same conditions can have CV values as great as 0.35 (Graham and Cruse 1981). PEP and anterior extreme position (AEP, the position of leg set-down at the end of swing) positions of all six legs of intact animals walking on horizontal or vertical planes or a horizontal beam have CV values ranging from 0.07 to 0.7, with one outlier having a CV of 4 and the average CV (without the outlier) being 0.27 (Cruse 1976). PEP and AEP of all six legs of intact animals freely walking on a glass plate have CV values ranging from 0.03 to 3.6, with four CVs >0.5, and
average CV values of 0.29 (all data) or 0.13 (excluding CVs >0.5) (Bässler 1972).

Remarkably, in none of this work is this high variability, particularly for a locomotory pattern (see following text), commented on. Similar situations are present in *Aplysia* feeding and the pyloric system of decapod crustacea. *Aplysia* feeding has been investigated for decades, but only recently have the great variability present in this motor pattern and the functional importance of this variability been explicitly examined (Breznina et al. 2005; Horn et al. 2004; Zhurov et al. 2005). In the pyloric system, although early work identified some of the neuronal connections giving rise to the modulation (Mulloney 1977), most work ignored an admittedly small modulation of the pyloric pattern by the much slower gastric mill pattern. However, work focusing on the motor expression of the extremely slow pyloric muscles shows that many of these muscles filter out the rapid bursting input of the pyloric motor neurons and express primarily or in some cases almost exclusively the gastric mill modulation (Morris et al. 2000). These observations raise the question of whether in other repetitive motor patterns, conscious or unconscious experimenters bias toward selecting experimental conditions in which only highly rhythmic activity is produced, or selecting only highly rhythmic data sequences to analyze, is occurring, and that a functionally equally or in some cases even more salient characteristic of the motor patterns, their variability, is thus being ignored.

*Extensor contraction during walking is highly variable—real or artifact?*

A first possibility is that the high variability in muscle contractions reported here arise from these data being obtained in single-legged animals, which lack feedback from adjacent legs (Bässler 1983; Cruse 1979, 1990; Graham 1985; Ludwar et al. 2005; Schmitz and Hassfeld 1989). However, as noted above, high variability is also present in intact animal walking. This observation does not prove that the same sort of variability is present in the two cases, but does indicate that variability per se is not attributable to the animals being in a single-legged condition. A second possibility is that the high variability arises from the slow extensor and common inhibitor axons not being stimulated or, if in some experiments the stimulation voltage was sufficient (see METHODS), their being coactivated with FETi instead of being stimulated to fire as they do in actual walking. This possibility is unlikely because experiments comparing muscle force when FETi alone or all three axons are stimulated shows that, under the experimental conditions used here, the other two scarcely alter FETi-induced force or movement (Guschlbauer, personal communication). A final possibility is that, as in *Aplysia* (Breznina et al. 2005; Horn et al. 2004; Zhurov et al. 2005), high variability is a physiological output of the walking neural network (see following text). If so, an intriguing possibility is that sensory input might match network variability to behavioral demand.

*Extensor contraction during walking is highly variable—a functionally advantageous combination of locomotion, active sensing, and crypsis?*

*Aplysia* feeding may be highly variable because *Aplysia* eat highly variable food. Because even less than ideal bites generally result in some food intake, high bite variability results in adequate food intake across a wide range of food types without maintaining the neural structures necessary to tailor feeding movements to individual food types (Horn et al. 2004). This strategy might seem impossible in locomotion because of the high cost of failure (falling) in locomotion. Indeed, none of the highly variable motor patterns mentioned earlier is locomotory, and we are unaware of reports of locomotory patterns with large variability. For instance, human walking has CV values of only 0.01 to 0.1 (Masani et al. 2002; Owings and Brabiner 2004; Terrier and Schutz 2003).

However, this lack may result from prior work being performed in predictable environments. For instance, two human locomotory patterns in which the limbs are used as active sensors would show great variability. The first is rock climbing, in which three limbs support the body while the fourth searches for new holds. The second is traversing swampy regions in which only parts of the surface can support human weight. In this case humans stand on a previously identified firm area and make repeated tentative stepping movements with one leg to identify where it is safe to step next. In both these cases locomotory-like movements with a wide variety of muscle contraction patterns (to bring the limb doing the active sensing to many endpoints) are repeatedly made.

These examples are particularly appropriate because, unlike the other animals in which locomotion has been well studied, *Carausius* live in trees, in which the majority of the volume is empty space and it is necessary to actively search for footholds. The large variability reported here may exist because during each swing phase *Carausius* uses its legs to actively search its immediate environment, and producing varying swing trajectories each step may be the most efficient strategy for finding footholds in a highly variable environment. Observations of free-moving animals support this suggestion. First, in dense vegetation the animals position their legs at rest not on one twig, but on several twigs and/or leaves. Second, in this environment the animals walk by clambering over the fens trated “surface” of vegetation and appear to make searching movements for new footholds with all six legs.

A second point relevant to this argument is that the energy savings associated with highly rhythmic locomotion (Dickinson et al. 2000) is likely less important in *Carausius* than in the other terrestrial species in which locomotion has been studied, which often travel long distances in search of food or mates. *Carausius* live among their food, primarily reproduce by parthenogenesis, and locomote over long distances only during food shortages. A third point is that *Carausius*’s primary defense against predators is hiding. Stereotyped rhythmic movements are likely highly indicative of animal movement and thus something that predators would have evolved to observe. If so, producing highly variable movements may help *Carausius* avoid detection. This hypothesis would also explain why the data visually identified as arising from “likely searches” and “likely steps” (Figs. 7 and 8, red and black data points) did not cleanly separate in most contraction measures—if clear steps and clear searches are extrema of a continuum, a clean separation would not be expected. A similar lack of clean separation between walking and searching movements is also present in another stick insect, *Cuniculina impi gira* (Bässler 1993), and this lack of separation is also consistent with modeling work showing that a single artificial
neural network can generate both step swings and searching-like movements (Dürr 2001).

These arguments together with the prior data from intact animal walking in the Comparison to prior work section earlier suggest that, although Carausius can produce typical hexapod walking, its normal walking may be instead highly irregular steps that both actively search the local environment and help the animal avoid detection. In this case, the high variability reported here would represent an advantageous adaptation to a slow-lane life in a highly fragmented and dangerous environment.

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