Tight or Loose Coupling Between Components of the Feeding Neuromusculature of *Aplysia*?

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Zhurov, Yuriy, Klaudiusz R. Weiss, and Vladimir Brezina. Tight or loose coupling between components of the feeding neuromusculature of *Aplysia*? *J Neurophysiol* 94: 531–549, 2005. First published March 9, 2005; doi:10.1152/jn.01338.2004. Like other complex behaviors, the cyclical, rhythmic consummatory feeding behaviors of *Aplysia*—biting, swallowing, and rejection of unsuitable food—are produced by a complex neuromuscular system: the animal’s buccal mass, with numerous pairs of antagonistic muscles, controlled by the firing of numerous motor neurons, all driven by the motor programs of a central pattern generator (CPG) in the buccal ganglia. In such a complex neuromuscular system, it has always been assumed that the activities of the various components must necessarily be tightly coupled and coordinated if successful functional behavior is to be produced. However, we have recently found that the CPG generates extremely variable motor programs from one cycle to the next, and so very variable motor neuron firing patterns and contractions of individual muscles. Here we show that this variability extends even to higher-level parameters of the operation of the neuromuscular system such as the coordination between entire antagonistic subsystems within the buccal neuromusculature. In motor programs elicited by stimulation of the esophageal nerve, we have studied the relationship between the contractions of the accessory radula closer (ARC) muscle, and the firing patterns of its motor neurons B15 and B16, with those of its antagonist, the radula opener (I7) muscle, and its motor neuron B48. There are two separate B15/B16-ARC subsystems, one on each side of the animal, and these are indeed very tightly coupled. Tight coupling can, therefore, be achieved in this neuromuscular system where required. Yet there is essentially no coupling at all between the contractions of the ARC muscles and those of the antagonistic radula opener muscle. We interpret this result in terms of a hypothesis that ascribes a higher-order benefit to such loose coupling in the neuromusculature. The variability, emerging in the successive feeding movements made by the animal, diversifies the range of movements and thereby implements a trial-and-error search through the space of movements that might be successful, an optimal strategy for the animal in an unknown, rapidly changing feeding environment.

INTRODUCTION

Complex adaptive behavior generally requires that many muscles and their neural control circuits coordinate their activities. In a complex neuromuscular system, to be sure, there will be many degrees of freedom, many redundant ways of reaching a given behavioral goal (Bernstein 1967). This high dimensionality can be exploited by the system for flexible achievement of the goal; at the same time, it makes it difficult to discern what neuromuscular variables the system is actually controlling to achieve it (for review, see Todoro 2004). However, although it is not clear what the control variables are, all approaches to this question agree that there are control variables, a relatively small number of dimensions—whether in the coordinates of the physical neuromuscular plant itself or those of the functional task—to which the degrees of freedom of the neuromuscular system are reduced in its operation (e.g., d’Avella et al. 2003; Scholz and Schöner 1999; Todoro 2004; Todoro and Jordan 2002; Zajac 1993; see DISCUSSION). In other words, the components of the neuromuscular system are functionally coupled.

A case in point is the neuromuscular system that mediates *Aplysia* consummatory feeding behaviors, the cyclical, rhythmic movements of biting, swallowing, and rejection of unsuitable food (Kupfermann 1974; for reviews, see Elliott and Susswein 2002; Kupfermann et al. 1997). The central structure in all of these movements is a hand-like grasping organ, the radula. In an ingestive behavior such as swallowing, the radula protracts from the mouth open, closes on food (seaweed), retracts to pull the food into the mouth, and opens to release the food into the esophagus. In egestive behavior (rejection), the phasing of the closing and opening of the radula is reversed with respect to its protraction and retraction so that the radula protracts closed rather than open, pushing swallowed material back out of the mouth. These complex, multidimensional movements of the radula are brought about by the combined contractions of the many muscles of the organ in which the radula is embedded, the buccal mass, in turn controlled by motor neurons driven by the motor programs of a single multitasking central pattern generator (CPG) in the buccal ganglia.

This description suggests that there must be a great deal of coordination and coupling between the neuromuscular components of the feeding apparatus if any functional movement is to be achieved. The recent structural and biomechanical work of Chiel and colleagues (Drushel et al. 1997, 1998; Neustadter et al. 2002a,b), which has revealed the very complex and intricate arrangement of the buccal mass, has reinforced this impression. It has thus generally been assumed that the activities of the various neuromuscular components in each type of feeding behavior are, if not completely stereotyped, then at least very tightly coupled. The degrees of freedom of the neuromuscular system are reduced to very few: if the contraction of one muscle alters, the contractions of the other muscles must immediately alter to compensate and maintain movements within the narrow, functional range (Brezina and Weiss 2000; Brezina et al. 2000a,b; Cropper et al. 2004; Hooper et al. 1999).

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Yet we have recently found that essentially all parameters of this neuromuscular activity—the phasing of the motor programs, motor neuron firing frequencies, muscle contraction amplitudes—vary over a large range, apparently randomly, from one cycle of the behavior to the next (Horn et al. 2004). The variability at all levels appears to be driven by the variability of the motor programs produced by the CPG. We have proposed that the CPG generates variable motor programs as part of a trial-and-error strategy that randomizes the feeding movements for optimal performance in an uncertain, variable feeding environment (Brezina et al. 2005; Horn et al. 2004).

How does this variability agree, however, with the tight coupling that is assumed to exist between the components of the buccal neuromusculature? We study this question here. We correlate the activities of two antagonistic buccal neuromuscular subsystems: the accessory radula closer (ARC, or I5) system, which closes the radula to grasp food (Cohen et al. 1978), and the radula opener muscle (I7) system, which opens the radula again (Evans et al. 1996). These muscles are certainly antagonists in the biomechanical sense: when their motor neurons are experimentally fired in semi-intact buccal mass preparations, they produce opposite movements (Orekhova et al. 2001). Furthermore, the two muscles tend to contract in opposite phases of the motor program on average (Zhurov et al. 2005). However, we find here that, in individual motor program cycles, the ARC and radula opener neuromuscular systems are not tightly coupled at all. This is not because such coupling cannot be achieved: the two ARC systems on the opposite sides of the animal, although not directly connected, are very tightly coupled in their simultaneous activity. These findings appear to require a significant reevaluation of our assumptions about the functional organization of motor control in the Aplysia feeding system.

**METHODS**

**Preparation**

The basic preparation used here was a reduced neuromuscular preparation designed for simultaneous recording from three separate neuromuscular subsystems: the two B15/B16-ARC systems on the opposite sides of the animal and the single central B48-radula opener system. A schematic diagram of the buccal ganglia and buccal mass is shown in Fig. 1. The two B15/B16-ARC systems are marked in red, the B48-opener system in blue, and buccal nerves that were recorded from or stimulated in these experiments in green.

The preparation combined, essentially, the standard B15/B16-ARC and B48-opener preparations that have been used in much previous work (e.g., Cohen et al. 1978; Evans et al. 1996; Weiss et al. 1979; for further references and most recent modifications, see Horn et al. 2004; Zhurov et al. 2005). Briefly, the preparation consisted of the two buccal ganglia, the two ARC (I5) and two radula opener (I7) muscles, and the connecting buccal nerves 3 through which the buccal motor neurons B15, B16, and B48 innervate the ARC and I7 muscles (Cohen et al. 1978; Evans et al. 1996). On one side of the preparation, the ARC muscle and the I7 muscles were left connected to the nerve; on the other side, just the ARC muscle. Because in the intact animal both of the centrally located I7 muscles are innervated through both buccal nerves 3 by the B48 neurons in both buccal ganglia, this dissection disconnected the I7 muscles from one of their B48 neurons (black scissor cut through the buccal mass in Fig. 1; but see following text). Similarly, the I7 muscles presumably remained innervated through the intact buccal nerve 3 by their motor neurons B4 and B5 (Evans et al. 1996; Warman and Chiel 1995) on one side, but were disconnected from those on the other side; however, we did not record from the B4 and B5 neurons in these experiments. The cerebral ganglion, connected to the buccal ganglia by the cerebral-buccal connectives, was also retained. The buccal ganglia (but not the cerebral ganglion) were desheathed.

In the bulk of the experiments analyzed in this paper, the side with the ARC and the I7 muscles was the right side of the animal and the side with just the ARC muscle was the left side, as shown in Fig. 1. However, we also included in the dataset a few older experiments in which only one ARC muscle was recorded, on the opposite side from the I7 muscles, and in some of these experiments the orientation was reversed. For this reason, we refer simply to side 1 (generally but not always, the right side, on which the ARC as well as the I7 muscles were recorded) and side 2 (generally, the left side, on which just the ARC muscle was recorded). We saw no obvious difference, arising from some inherent asymmetry in the animal or the asymmetrical arrangement of the final preparation, in the activities of the B15/B16-ARC systems on the two sides.

Because we were concerned that the disconnection of the I7 muscles from their B48 (and B4/B5) neurons on one side could have affected our conclusions (see RESULTS), we performed several supple-
mentary experiments in which we did not make the black scissor cut in Fig. 1. The I7 muscles remained fully innervated on both sides; we recorded the I7 muscles and (although now mechanically more awkward) one or both of the ARC muscles.

The three muscles (the two I7 muscles being treated as one mechanical unit) were pinned out in two separate subchambers (one containing both the ARC and I7 muscles, the other just the ARC muscle) and each connected to an isotonic transducer (Model No. 60–3000, Harvard Apparatus, Holliston, MA) to measure the length of the muscle with a light counterbalancing load. Two of the relevant motor neurons in the buccal ganglia—in different experiments either the two opposite B15s, the two opposite B16s, B48 on the side of the intact I7 innervation and the opposite B15, or B48 and the opposite B16 (or, in the supplementary experiments with the fully intact I7 innervation, the two opposite B48s)—were impaled with standard intracellular microelectrodes and their membrane voltage was monitored with an intracellular amplifier (Axoclamp 2A/B, Axon Instruments, Union City, CA). Electrical activity in two buccal nerves, namely the I2 nerve and one of the buccal nerves 2 (control recordings showed that motor-program activity appeared similarly in both buccal nerves 2) (for nerve nomenclature, see Cohen et al. 1978; Hurwitz et al. 1994; Scott et al. 1991), was recorded differentially through suction electrodes connected to an extracellular amplifier (Differential AC Amplifier Model 1700, A-M Systems, Carlsborg, WA). All signals were sampled and recorded simultaneously by a computer using Digidata 1322A data-acquisition hardware and pCLAMP 8/9 software (Axon Instruments).

The extracellular amplifier, under the control of a separate stimulator (Grass S48/S88, Astro-Med, West Warwick, RI), was also used to stimulate the esophageal nerves. Either one or the other esophageal nerve was stimulated or usually both, drawn into separate suction electrodes but stimulated identically in parallel. These three stimulation variants gave essentially identical results (i.e., stimulation of either esophageal nerve was sufficient to elicit normal, complete programs of similar strength simultaneously in both buccal ganglia) except that stimulation of both nerves sometimes appeared to elicit faster programs, with a shorter cycle period, than stimulation of just one nerve. The esophageal nerve stimulation consisted of a long train of regular voltage pulses with parameters adjusted so as to elicit identifiable motor programs (see following text) at moderately frequent intervals while the stimulation continued. The individual voltage pulses were 7–15 ms in duration, 7–15 V in amplitude, delivered at 1–3 Hz. Three-minute blocks of this stimulation, separated by 7-min rest periods, were repeated as long as the motor programs continued to be elicited.

Experiments were done either at ~17°C or at room temperature (21–24°C); no obvious differences in the phenomena of interest here were observed and the results have been pooled.

Data analysis

Motor programs were identified by the presence of a characteristic coordinated pattern of electrical activity in the I2 nerve and buccal nerve 2, namely first a burst of at least moderately intense activity in the I2 nerve, then, beginning soon after the end of the I2 nerve burst, a characteristically-shaped burst of intense activity in buccal nerve 2 (see Fig. 2) (for previous use of these criteria see, e.g., Horn et al. 2004; Hurwitz et al. 1996; Jing and Weiss 2001, 2002; Kabotyanski et al. 2005).

FIG. 2. Typical 3-min block of motor programs elicited by esophageal nerve stimulation. Simultaneous recording of (top to bottom) the length of the two ARC muscles and of the opener muscle, membrane voltage of the motor neurons B16 and B48 (recorded intracellularly), and electrical activity in the I2 nerve and buccal nerve 2 (recorded extracellularly). The 3-min period of esophageal nerve stimulation is indicated at the bottom. Bursts of activity in the I2 nerve and buccal nerve 2, respectively, were taken to define the protraction and retraction phases of each program (gray rectangles; see METHODS). Note that muscle length is plotted so that decreasing length—increasing contraction—is upward.
al. 1998; Morgan et al. 2002; Morton and Chiel 1993a; Nargeot et al. 1997; Proekti et al. 2004; Zhurov et al. 2005). The protraction phase of the program was defined to be coincident with the I2 nerve burst; the retraction phase was defined as lasting from the end of the I2 nerve burst to the end of the buccal nerve 2 burst. The motor program was then the unit of these two phases, and a complete cycle was defined as the motor program together with the preceding interprogram interval (see Fig. 2). Although other program features—for example, characteristic bursts of motor neuron B15, B16, or B48 firing (Figs. 2, 4, 5, and 10)—could be seen in the other recording channels, the definition of motor programs in terms of the I2 nerve and buccal nerve 2 bursts was primary. When either burst was very weak or absent, no program was identified. When a program could not be thus identified, yet the other recording channels showed a strong program, the entire block of stimulation was rejected. Furthermore, only those blocks were accepted that had four or more identified motor programs and at least some contraction in each of the muscles. When a block was accepted, all motor programs in it were analyzed. Altogether, 2,612 programs in 276 blocks from 28 preparations were accepted for further analysis as our basic dataset in this paper. (The supplementary experiments with the fully intact I7 innervation yielded 554 further programs in 39 blocks from 4 preparations.) However, because not all variables—in particular, only two of the motor neurons—were recorded in each experiment, different analyses reported here use different, and differently sized, subsets of the basic dataset (see figure legends).

Initial processing of the raw records was done in Clampfit (Axon Instruments). The programs were identified and the beginning and ending times of their protraction and retraction phases were marked by eye. This appeared to be sufficiently reliable as the changes in nerve activity were usually quite abrupt (e.g., Fig. 2). Motor neuron B15, B16, and B48 spike times were automatically tabulated using the threshold event detection module of Clampfit 9.

Subsequent processing of the data, its collation across multiple blocks and preparations, and all statistical analysis were done in Mathematica 4/5 (Wolfram Research, Champaign, IL) or in Sigma-Plot 8 (Systat Software, Point Richmond, CA). For some analyses, the motor neuron B15, B16, and B48 spike times were converted to instantaneous firing frequency waveforms, assigning to each time point in an interspike interval the reciprocal of the duration of that interspike interval. For comparison between blocks and preparations, each block-long frequency waveform was then normalized by the 95th percentile of the values in the waveform to neutralize the effect of spuriously high frequencies. This rescaled 95% of the waveform to between 0 and 1. Similarly, the block-long ARC or opener muscle length waveform, which in this paper is always plotted inverted so that decreasing length, or increasing contraction, is upward (see, e.g., Fig. 2), was rescaled to between 0 (minimal contraction, i.e., maximal length, in the block) to 1 (maximal contraction, i.e., minimal length, in the block). Further details of the analysis are given in RESULTS and in the figure legends.

RESULTS

All data in this paper are drawn from one basic type of preparation, a reduced neuromuscular preparation of the buccal ganglia and the buccal mass (Fig. 1) in which we could simultaneously record the activities of three separate buccal neuromuscular subsystems: the ARC muscle and its two motor neurons B15 and B16 (Cohen et al. 1978) on one side of the animal, the ARC muscle and its motor neurons B15 and B16 on the opposite side of the animal (the two B15/B16-ARC systems are marked in red in Fig. 1), and the core component of the single central radula opener muscle system, the paired I7 muscle and its principal motor neuron B48 (Evans et al. 1996) (the B48-opener system is marked in blue in Fig. 1). Through-out, we examined the activities of these three neuromuscular systems in motor programs generated endogenously by the buccal feeding CPG. The rhythmic cycling of the motor programs was, of course, very clearly expressed in the motor neuron firing and muscle contractions of the three neuromuscular systems themselves (see, e.g., Fig. 2, top five records). For independent demarcation of the motor programs, however, we used standard criteria, often used for this purpose in previous work (see METHODS), to define the protraction and retraction phases of each program from the extracellularly recorded electrical activity of two buccal nerves, the I2 nerve and buccal nerve 2 (green in Fig. 1), respectively. We define a “motor program” as one unit of these two phases and a complete “cycle” as a motor program together with the preceding interprogram interval (see Fig. 2, bottom two records).

Although the structure of the motor programs was produced endogenously by the CPG, the CPG, which does not cycle spontaneously, still needed to be triggered into activity. To do this, again as in previous work (e.g., Chiel et al. 1986; Horn et al. 2004; Morgan et al. 2002; Proekti et al. 2004; Zhurov et al. 2005), we electrically stimulated another of the buccal nerves, the esophageal nerve (green in Fig. 1; the stimulation was usually bilateral: see METHODS). In our standard protocol, we stimulated the nerve with regular brief shocks delivered at moderate frequency (1–3 Hz) continuously for 3 min. Figure 2 shows one such “block” of stimulation. In this case, we recorded (top to bottom) the contractions of the two ARC muscles and of the opener (I7) muscle, the membrane voltage—revealing both spikes and subthreshold synaptic potentials—of the ARC motor neuron B16 and the opener motor neuron B48, and the electrical activity in the I2 nerve and buccal nerve 2. As can be seen, multiple motor programs were typically elicited during the 3-min block—in this case, 22 distinct programs. After a rest period of 7 min, another block was recorded. Altogether, we collected, as our basic dataset in this paper, 2,612 programs in 276 blocks (~9.5 programs/block on average) from 28 preparations satisfying our criteria (see METHODS) for further analysis. However, because not all variables—in particular, never more than two of the six relevant motor neurons B15, B16, and B48—were recorded in each preparation, different analyses presented below use different, smaller subsets of this basic dataset (see figure legends for details).

Contractions of the two ARC muscles are similar, those of the opener very different

Examination of Fig. 2 reveals again the extreme variability that was already quantified from such blocks of esophageal motor programs by Horn et al. (2004). Even though the esophageal nerve stimulation is constant and regular throughout the block, essentially all parameters of the neuromuscular activity—the phasing of the motor programs, the motor neuron firing frequencies and patterns, the muscle contraction amplitudes—vary greatly from one cycle to the next. This variability is, to a first approximation, random (Horn et al. 2004).1

1 The variability is clearly not completely random in at least two ways. First, there are clear systematic trends in parameter values over successive programs in the block that are often completely masked by the large cycle-to-cycle variability in any single block but are revealed when many blocks are averaged together (Zhurov et al. 2005). Second, in this work we observed that a parameter sometimes exhibited a distinct temporal metastructure that recurred
Given this extreme variability, it is striking in Fig. 2 how similar the two ARC contraction waveforms are to each other throughout the block. Although the size and shape of the contraction varies greatly from cycle to cycle, this happens in the same way in parallel in both ARC muscles. In contrast, the shape of the opener contraction waveform does not appear to be related in any obvious way to those of the two ARC waveforms. (Further examples can be seen in Figs. 4A, 5A, and 10A.)

To begin to quantify these relationships, we first computed simply the pointwise correlations between the entire block-long contraction waveforms. Figure 3A shows representative correlation plots between the two ARC waveforms (right) and between one of the ARC waveforms and the opener waveform (left) from 7 blocks in one preparation. Each point in these plots represents 100 ms of contraction waveform. The best-fit linear regression lines are shown and the coefficient of determination, $R^2$, is given in each case. Figure 3B is then a histogram of the $R^2$ values from 168 such ARC-ARC (black bars) and 171 ARC-opener (gray bars) plots (each from one block). It is clear that the two ARC waveforms were generally very well correlated (the median ARC-ARC $R^2$ value in Fig. 3B is 0.86), whereas the ARC and opener waveforms were essentially uncorrelated (the median ARC-opener $R^2$ value is 0.03). The correlation between the ARC waveforms was always positive, and usually very close to an identity as in Fig. 3A.

The strong correlation between the two ARC muscles immediately suggests a tight functional coupling between them during the operation of the buccal neuromusculature. The lack of correlation between the ARC and opener muscles, on the other hand, is not especially informative. Because the ARC and opener muscles are antagonists, we should certainly not expect to find a positive correlation between their contractions—if anything, a negative correlation—but quite possibly there might be some simple linear relationship at all even if the contractions were, in fact, tightly coupled. To establish that the ARC and opener muscles are not tightly coupled, we have to analyze the data in a more sophisticated way. We will do so later in RESULTS. First, we will examine further the strong correlation between the two ARC systems on the opposite sides of the animal.

**ARC motor neurons B15 and B16 are synchronized in their activity on the two sides of the animal**

The ARC muscle contraction waveform is produced by the firing pattern of the two motor neurons B15 and B16 through a neuromuscular transform (Brezina et al. 2000a) that is highly nonlinear but precisely repeatable and essentially deterministic (Horn et al. 2004; Zhurov et al. 2004). The similarity of ARC waveforms therefore strongly suggested a similarity—a syn-
chronization—of the motor neuron B15 and B16 firing patterns on the two sides of the animal.

Figure 4 shows an example in which we compared the firing patterns of the two motor neurons B15. Underlying the similar ARC contraction waveforms (Fig. 4A, middle two records) were indeed very similar firing patterns of the two motor neurons (bottom two records). A more detailed examination of the subthreshold membrane voltage range (Fig. 4B) revealed furthermore a clear synchrony of the postsynaptic potentials (PSPs) received by the two motor neurons, both of putative EPSPs (*) and putative IPSPs (**).2

Figure 5 shows another example in which we compared the firing patterns of the two motor neurons B16. Again, there was a striking similarity of the two firing patterns, at the overall level of the entire block of motor programs (Fig. 5A), through the level of individual motor programs (Fig. 5B), down even to the level of individual spikes and putative PSPs (Fig. 5C).

At the level of individual spikes, many spikes in the two motor neurons appeared to be precisely synchronized with each other (Fig. 5C, ↓↓), whereas some clearly were not (↓). Motor neuron B16, unlike B15, fired a sufficient number of spikes for us to be able to examine the degree of spike-level synchrony quantitatively by constructing crosscorrelograms. Figure 6A shows a crosscorrelogram constructed from all motor neuron B16 spikes recorded in the protraction phases of 911 motor programs in nine preparations. A complete lack of correlation—complete independence of the timing of the spikes in the “target” motor neuron B16 of the timing of the spikes in the “reference” motor neuron B16—would be indicated by a completely flat crosscorrelogram (as in Fig. 6C). The presence in Fig. 6A of distinct peaks, on the other hand, shows that the timing of the spikes is not independent. The large central peak, in particular, contains the spikes fired by the target motor neuron B16 preferentially around (within 10 ms, the bin width) the time of a spike in the reference motor neuron B16. To estimate the strength of the crosscorrelation, we computed the ratio \( a/(a + b) \) (see labels in Fig. 6A), where \( a \) is the height of the central peak above the mean firing frequency—the expected height of a completely flat crosscorre-

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2 We did not attempt any systematic analysis of the nature of the PSPs as it was not important for the purposes of this paper. However, some PSPs were always associated with an overall depolarization of motor neuron B15, in particular during the retraction phase (Fig. 4B, *) and so may have been EPSPs. Another class of PSPs, prominent in both motor neurons B15 and B16 immediately after the end of retraction, during the interprogram interval and continuing into the next protraction phase (Figs. 4B, **, 5C, **, and 7A), presented as a relatively rapid hyperpolarization followed by a slower depolarizing recovery, and so were probably IPSPs. Furthermore, in motor neuron B16 at least, these IPSPs could be reversed by hyperpolarization of the neuron.
logram—of the target motor neuron B16, $b$ (equal to 9.71 Hz in Fig. 6A: - - -). This gave an overall correlation strength of 0.51 for Fig. 6A.

The three individual plots below Fig. 6A show the protraction-phase crosscorrelograms constructed from three individual preparations. As was to be expected on general statistical grounds, preparations that had a low mean firing frequency of the motor neurons B16 tended to exhibit higher correlation strengths (e.g., left individual plot) and preparations that had a high mean firing frequency, lower correlation strengths (right individual plot). Linear regression of the correlation strengths, $s$, computed from the nine individual preparations against the mean firing frequency, $f$, yielded a best-fit linear relationship of $s = 0.89 - 0.031f$ Hz, with $R^2 = 0.53$. The mean ± SD of the individual correlation strengths was 0.50 ± 0.089, similar to the overall pooled value of 0.51.

Figure 6B shows another crosscorrelogram, as in Fig. 6A except constructed from all motor neuron B16 spikes during the retraction phases of the 911 programs. In retraction,
sharp contrast to protraction, there was very little correlation in the timing of the motor neuron B16 spikes: the overall correlation strength was only 0.092.

Finally, the motor neurons B16 sometimes fired spontaneously during the 7-min rest intervals between the blocks of esophageal nerve stimulation (e.g., Fig. 5A). Figure 6C shows a crosscorrelogram constructed from such spontaneous firing during 21 interblock intervals in four preparations. Here, even though each of the two B16 motor neurons fired at comparably high frequency and regularly, as shown by the distinct peaks in each of their two autocorrelograms (Fig. 6C, insets), there was absolutely no correlation between them. With the slight differences in their firing frequencies that necessarily always existed, their firing patterns gradually drifted against each other through all possible phase combinations.

Why do the spikes in the two motor neurons B16 lock synchronously with respect to each other in protraction (Fig. 6A) but not in retraction nor in the interblock interval (Fig. 6, B and C)? Almost certainly, it is because they are synchronized by the large PSPs—apparently IPSPs—that arrive in protraction (e.g., Fig. 5C, **) but not in retraction nor in the interblock interval, when motor neuron B16 receives no obvious synaptic input at all (Fig. 5, A and C).

Although the IPSPs synchronized the spikes in protraction, they were themselves best examined in the immediately preceding phase, the interprogram interval, where they were so large and frequent that (although other factors may also have contributed) they suppressed spiking altogether (see Fig. 5, A–C). (Hence we could not construct a spike crosscorrelogram for the interprogram interval in Fig. 6.) Figure 7A shows an example in which we compared the subthreshold voltage waveform of the two motor neurons B16 in the interprogram interval. Most of the IPSPs (peaks marked by ●) were clearly synchronized, although an occasional one was not (†). Figure 7B shows a crosscorrelogram constructed from all IPSPs recorded in 134 interprogram intervals in eight preparations. There was, indeed, a good overall correlation in the timing of the IPSPs in the two motor neurons; the correlation strength,
computed in the same way as for the spike crosscorrelograms in Fig. 6, was 0.72.

Finally, all of the synchronization between the two B15/B16-ARC systems on the opposite sides of the animal was disrupted by cutting of the commissure between the two buccal ganglia (gray scissor cut in Fig. 1). Figure 8 shows this for the ARC muscle contractions and the firing of the two motor neurons B16. When the commissure was cut, each buccal ganglion generated, apparently, its own independent motor program, desynchronized from that on the other side and drifting, with its motor neuron firing patterns and muscle contractions, through all possible phase combinations relative to it. [Consequently, crosscorrelograms such as those in Figs. 6A and B became flat (not shown).]

Two B15/B16-ARC systems are not directly connected

All of the findings in the last section could be explained if the B15/B16-ARC systems on the two sides of the animal were directly connected—if, for example, each of the motor neurons B15 and B16 were connected by an electrical or excitatory chemical synaptic connection to its counterpart on the other side, so that a spike on one side immediately elicited a spike also on the other. However, Fig. 9 shows that this is not the case. A burst of spikes (triggered by current injected through the intracellular recording electrode) in each motor neuron B15 (Fig. 9A) or B16 (B) contracted the ARC muscle only on its own side, with no response at all in the ARC muscle or in the counterpart motor neuron B15 or B16 on the other side. [Numerous previous experiments, beginning with those of Cohen et al. (1978), have shown that there is no connection between the motor neurons B15 and B16 on the same side.] Finally, during ongoing esophageal motor programs, injection of steady hyperpolarizing current to silence either motor neuron B15 or B16 had no obvious effect on the firing pattern of the other motor neuron B15 or B16 or the contractions of the other ARC muscle (not shown).

Two opener motor neurons B48 are also synchronized

There are in fact two I7 opener muscles (Fig. 1), but they are adjacent to each other, coupled mechanically as well as elec-
trically through gap junctions, and both appear to be innervated by each of their motor neurons (Evans et al. 1996): they constitute, functionally, a single central unit. The entire unit is, however, innervated by two separate motor neurons B48, one in each buccal ganglion (Fig. 1). In a special set of supplementary experiments (see following text) we were able to record from both motor neurons B48 simultaneously. Figure 10 shows a block of esophageal motor programs from one of these experiments. Clearly, as with the ARC motor neurons B15 and B16, there was a marked similarity between the firing patterns of the two motor neurons B48, at the overall level of the entire block of motor programs (Fig. 10A, bottom two records) as well as the level of the individual programs (Fig. 10B).

In more detail, however, some of the synchronization between the firing patterns of the two motor neurons B48 had its origin in their common entrainment by the regular voltage

FIG. 8. The 2 B15/B16-ARC systems are desynchronized by cutting of the buccal commissure. A: then excerpted in B: part of a block of esophageal motor programs as in Fig. 5 except after the buccal commissure had been cut (gray scissor cut in Fig. 1). The bursts of activity in the 2 buccal nerves 2 (not shown) were also desynchronized. Similar desynchronization, of ARC muscle contractions, B16 firing, or buccal nerve 2 activity, was observed in 3 other preparations.

FIG. 9. The 2 B15/B16-ARC systems are not directly connected. A: a burst of spikes in B15(1) (each spike was elicited by injection of a brief depolarizing current through the intracellular electrode) produced a contraction of the ARC(1) muscle but no response at all in B15(2) or ARC(2). Conversely, a burst of spikes in B15(2) contracted the ARC(2) muscle but produced no response in B15(1) or ARC(1). B: as in A but for B16. Similar results were obtained in 4 other preparations.
pulses applied to the esophageal nerve. Each voltage pulse elicited a prominent EPSP in each motor neuron B48 (e.g., Fig. 10B, ↑) which tended to group the following spikes into a burst of higher frequency. [This was not the case in motor neuron B16 (see text footnote 3) or, apparently, B15.] Because the esophageal nerve stimulation is believed to mimic natural sensory stimuli arriving during feeding (see DISCUSSION), such common entrainment may represent an entirely physiological mechanism of synchronization of the two motor neurons. On the other hand, it might be unphysiological. To avoid possibly spurious correlations due to the entrainment, therefore, we did not construct crosscorrelograms for the motor neurons B48 as we did for B16. Instead, to capture the overall similarity of the firing patterns, we simply correlated pointwise, in the same way as we did for the ARC and opener contraction waveforms in Fig. 3, the entire block-long instantaneous firing frequency waveforms of the two motor neurons B48. This correlation is not so sensitive to the exact timing of spikes but remains sensitive to the general shape and especially the amplitude of the frequency waveforms. Figure 10C shows the frequency waveforms computed from the firing patterns of the two motor neurons B48 in the block in Fig. 10A. Figure 11A shows a representative correlation plot of such a pair of waveforms. Each point represents 100 ms. The best-fit linear regression line is shown and the value of $R^2$ is given. Figure 11B is a histogram of the $R^2$ values from 39 such plots (each from one block) from four preparations. The median $R^2$ value is 0.63. Clearly, there was a considerable degree of correlation between the firing patterns of the two motor neurons B48, albeit less than between the two ARC contraction waveforms or, perhaps, the firing patterns of the two motor neurons B15 or the two motor neurons B16.

Finally, as with the motor neurons B15 and B16 in Fig. 9, when we fired one motor neuron B48, we observed no response in the other. However, as expected, both motor neurons B48 contracted the opener muscle; when both were fired together, the contractions were approximately twice as large as when only one or the other was fired alone (not shown).

Correlations between components of the B15/B16-ARC and B48-opener systems in different program phases

We now return to the question of how well the ARC and opener systems are coupled. We found no correlation between whole ARC and opener contraction waveforms (Fig. 3), but as we noted, this could simply mean that we do not know what quantitative form a coupling between them would take. How can we answer this question more directly?
tor program or successive program pair. The best-fit linear regression line (gray line) is shown where any significant correlation was found and the value of $R^2$ is given.

As expected from the similarity of the whole ARC waveforms in Fig. 3, there was a good correlation between the mean contraction amplitudes of the two ARC muscles in protraction (Fig. 12A, left) as well as retraction (middle). Again, however, there was essentially no correlation between the mean contraction amplitudes of the ARC and the opener muscle, in protraction (Fig. 12A, right). [The opener muscle does not contract much at all in retraction (see, e.g., Fig. 4A) (Zhurov et al. 2005), so this correlation could not be performed.]

There was also only a weak correlation between the mean amplitudes of the contraction of the same ARC muscle in the protraction and the immediately following retraction phases of the same motor program (Fig. 12B).

Finally, first return maps—that is, plots of the amplitude in program $n + 1$ against that in program $n$—showed only weak or moderate correlations between the mean contraction amplitudes of the ARC muscle in successive protraction (Fig. 12C, left) and retraction (middle) phases as well as of the opener muscle in successive protraction phases (right).

Figure 13 uses these muscle correlations and analogous correlations of the motor neuron firing frequencies to plot a diagram of the web of interrelationships among the components of the B15/B16-ARC and B48-opener systems. Each number, and the thickness of the corresponding arrow, indicates the $R^2$ value from a correlation like those in Fig. 12. The general features of the plot can be summarized as follows. Internally among the components of each B15/B16-ARC system in any particular program (bright red symbols and arrows), and among the components of the B48-opener system although in this case there were few components to correlate (bright blue symbols and arrows), there were only weak correlations. With one interesting exception (see following text), there were only moderate correlations between each of these components in that program and in the following program (gray symbols and arrows). The weakness of these two types of correlations, between different components of the system in the same cycle and the same component in successive cycles, reflects the large random variability described by Horn et al. (2004). In striking contrast to these weak correlations, there were very strong correlations between each component of the B15/B16-ARC system and the simultaneous activity of its counterpart on the other side of the animal (dark red symbols and arrows), and similarly, albeit to a lesser degree, in the B48-opener system (dark blue symbols and arrow). However, there was essentially no correlation at all between the simultaneous activities of the components of the B15/B16-ARC systems and those of the B48-opener system (two vertical black arrows, so thin as to be nearly invisible, and so emphasized by the large open arrows).

The one exception to the generally weak correlations of the same component in successive cycles in Fig. 13 was the strong correlation of motor neuron B16 firing in retraction, with $R^2 = 0.85$. In other words, the mean firing frequency of motor neuron B16 tended to be very similar in successive retraction phases. Because B16 receives, apparently, no synaptic input at all in retraction (Fig. 5C), its firing in each successive retraction phase probably reflected simply the spontaneous firing of the motor neuron, such as was seen also between the blocks of esophageal nerve stimulation (Fig. 5A). Contrast this with...
protraction, with its prominent IPSPs, where the correlation of B16 firing in successive cycles was much weaker, with $R^2 = 0.37$. The exceptional low variability of motor neuron B16 firing in retraction is thus in fact very consistent with the conclusion of Horn et al. (2004) that it is the activity of the CPG, reaching the components of the system through their mutual interconnections, that generates the variability.

Distances between the shapes of the ARC and opener contractions

The results in the previous section again support the idea of a tight coupling between the two ARC systems but only loose coupling between them and the opener system. However, the logical difficulty remains. If good correlations had appeared between the mean motor neuron firing frequencies and mean contraction amplitudes of the ARC and opener systems, this would have been significant. But since correlations did not appear, it can always be argued that the mean firing frequencies and contraction amplitudes—or any other parameter that fails to show good correlations—were simply not the right parameters, those related to the actual control variables of the system, to examine.

For our final analysis, we therefore employed a different type of argument. Say, hypothetically, that there is tight coupling between the contractions of the ARC and opener muscles. We do not know what quantitative form this coupling takes, but we know that it is tight, meaning that a particular shape of contraction of the ARC muscle is matched by a particular shape of contraction of the opener muscle. There is, of course, great variability in the shapes of both contractions in successive motor programs. However, if we find two programs in which the ARC contractions have a similar shape, the shapes of the opener contractions in those two programs should be similar also. More formally, each contraction shape sampled at $n$ time points can be thought of as a point in an $n$-dimensional space. In each of the cartoons in Fig. 14A, right, the ARC contraction shape space is represented by a red circle and the opener space by a blue circle. If the tight coupling exists, contraction shape points that lie close to each other in one space should correspond to points that lie close to each other in the other space. Mathematically, there should be a one-to-one mapping between the two spaces. The first cartoon, in Fig. 14A1, illustrates this scenario.

Does such a mapping in fact exist? In Fig. 14 we selected, from a pool of 1,624 motor programs from our basic dataset, two programs at random, 10,000 times over. For each of these 10,000 program pairs, we computed $d_{\text{ARC}}$, the root mean square difference between the contraction shapes of one of the ARC muscles (results with either ARC muscle were essentially identical) in the two programs, and $d_{\text{opener}}$, the corresponding difference between the opener contraction shapes. From its formula (see Fig. 14 legend), the root mean square difference can be seen as a normalized Euclidean distance between two points in an $n$-dimensional space. Three representative program pairs, with the ARC contractions shown in red and the opener contractions in blue, are shown in Fig. 14A, left. All
10,000 pairs of corresponding differences ($d_{ARC}$, $d_{opener}$) are plotted in Fig. 14B. There were, in fact, cases where, when the two ARC contractions were similar, so were the two opener contractions, as illustrated in Fig. 14A1. But these cases were rare. Much more common were cases where the ARC contractions were similar, but the opener contractions were very different (Fig. 14A2), or, conversely, where the opener contractions were similar but the ARC contractions were different (Fig. 14A3). Over all of the 10,000 program pairs in Fig. 14B (both gray and black points), there was no correlation at all between $d_{ARC}$ and $d_{opener}$ ($R^2 < 0.01$).

When many blocks of esophageal motor programs are averaged together to overcome the random variability, it is found that successive programs within the block exhibit a progressive development in their parameters, including their ARC and opener muscle contraction shapes, toward a mature form that is reached after perhaps five programs (Zhurov et al. 2005). We therefore asked whether the mature ARC and opener contractions were any better correlated. However, we found no correlation either when we considered only that subset of the 10,000 program pairs in which both programs were the 6th or later in their block (Fig. 14B, black points). (Neither was there any correlation if the 10th or later programs were considered, or programs occurring later than some given time, such as 1 or 2 min, after the beginning of the 3-min block.)

Finally, we were concerned by one point. The surgical procedure that we used in our basic set of preparations rendered the innervation of the opener muscle asymmetrical by cutting off one side of the innervation of the muscle (Fig. 1, black scissor cut). This would only have distorted our results significantly if a coupling had existed between the ARC contractions and the contractions of the fully innervated opener muscle but not those of the opener muscle innervated only from one side. This in turn would have required that the contractions elicited by the motor neurons B48 on the two sides were different but always complementary, themselves coupled so that the result could be coupled to the contractions of the ARC muscle. Contrary to this scenario, we found in Figs. 10 and 11 that the firing patterns of the two motor neurons B48 were similar. To be sure on this point, however, we collected and analyzed a supplementary dataset of 554 motor programs from four preparations in which we kept the innervation of the opener muscle fully intact on both sides of the animal. (The comparison of the two motor neurons B48 used this dataset.) Drawing from this dataset 1,000 random program pairs and computing the differences ($d_{ARC}$, $d_{opener}$), we found that these, too, were essentially uncorrelated ($R^2 = 0.02$; not shown).

Altogether, then, we found no evidence for any tight coupling between the ARC and opener muscle contractions.

**DISCUSSION**

The basic finding of this paper can be very simply stated. Whereas there is tight coupling between the activities of the corresponding neuromuscular components on the two sides of the animal, in particular between the two ARC systems, there appears to be no such coupling between the ARC system and its presumed antagonist, the opener system.

**Tight coupling between the two sides of the animal**

In the esophageal motor programs that we have studied here, we have found striking similarity between the firing patterns of the two motor neurons B15 (Figs. 4 and 13), between the firing patterns of the two motor neurons B16 (Figs. 5, 6, and 13), and, as a consequence, between the shapes of the contractions of the two ARC muscles that these motor neurons innervate (Figs. 2–5, 10, 12, and 13), on the opposite sides of the animal. In the two motor neurons B16, it is clear that the firing patterns are...
well synchronized down even to the level of the individual spikes (Fig. 6). Yet there is no direct connection between the two motor neurons B16, the two motor neurons B15, or apparently any other part of the two B15/B16-ARC systems (Fig. 9). The B15/B16-ARC systems on the two sides of the animal are separate neuromuscular circuits. Their synchronization is achieved, almost certainly, by the synchronous PSPs, in particular a class of prominent IPSPs, that they receive (Fig. 7). These presumably originate in some common element in the buccal feeding circuitry that, in this respect, serves as a synchronizing center for the two sides of the animal. This element could be a single neuron that has equally strong connections to both sides, or (because unpaired neurons are rare) a pair of such neurons, one in each buccal ganglion, or a pair of neurons with unequal connections to the two sides, but strongly coupled to each other. A number of neurons have been described with such properties (although in most cases their connections to B15 and B16, specifically, were not tested), including B4 and B5 [known to inhibit both B15 and B16 (Cohen et al. 1978)], B20 [known to excite B16 (Teyke et al. 1993)], B51 and B52 (Plummer and Kirk 1990), B64 (Hurwitz and Susswein 1996), and B63 and B34 (Hurwitz et al. 1997).

The firing patterns of the two opener motor neurons B48 also appear to be synchronized on the two sides of the animal, although perhaps less strongly than those of the ARC motor neurons (Figs. 10, 11, and 13). In this case, the mechanism of the synchronization is less clear. The two motor neurons B48 are not mutually connected, but any PSPs that they receive are not especially prominent. In this case, furthermore, the synchronization may have been partly due to the common entrainment of the motor neurons B48 by our esophageal nerve stimuli in these experiments (Fig. 10B).

Loose coupling between the ARC and opener systems

We have probed the coupling between the ARC and opener systems, in particular between the shapes of the ARC and opener muscle contractions, with complementary direct and indirect approaches. Directly, we have correlated whole contraction waveforms (Fig. 3) or selected parameters of them (Figs. 12 and 13). This approach suffers from the fact that its results are only interpretable if a correlation actually appears—it cannot prove a negative. Indirectly, we have examined the overall mapping between the spaces of ARC and opener.
opener contraction shapes (Fig. 14). This approach, conversely, can easily rule out the existence of a correlation—as it did here—but if a correlation did exist, its particular properties would not immediately be obvious. Together, the similar answers given by both approaches strongly suggest that there is essentially no correlation between the ARC and opener contractions in the esophageal motor programs.

The ARC and opener muscles are indeed antagonists in the biomechanical sense that when their motor neurons are fired, they produce opposite movements (Orekhova et al. 2001). Furthermore, when in a previous study we averaged together a large number of esophageal motor programs similar to those recorded here, the two muscles were found to behave antagonistically on average, contracting, for instance, in opposite phases of the program so that when the ARC muscle was contracted the opener muscle was relaxed and vice versa (Zhurov et al. 2005). What our results here mean is that this average behavior becomes essentially undetectable due to a large variability in the relationship between the contractions of the two muscles from one cycle to the next, another manifestation of the random cycle-to-cycle variability described by Horn et al. (2004).

**Functional implications of tight and loose coupling**

Horn et al. (2004) found large, apparently random variability, between values of different parameters in the same cycle as well as the same parameter in successive cycles, in essentially all low-level parameters of the neuromuscular activity involved in *Aplysia* feeding behavior such as the motor program phase durations, motor neuron firing frequencies, and individual muscle contraction amplitudes. We have seen this variability again here in the general weakness of the correlations—except those between the two sides of the animal—in Fig. 13. In other words, the low-level parameters are only loosely coupled to each other. The major finding that we have added here is that the loose coupling extends even to higher-level features of the organization of the neuromuscular system such as the coordination between entire subsystems within it, which, especially if they are antagonistic subsystems, have always been assumed to be necessarily tightly coupled if functional behavior is to be produced at all.

Indeed, under these circumstances, how can functional behavior be produced? Recapitulating and enlarging the argument of Horn et al. (2004), we consider three scenarios of the functional organization of the neuromuscular system, illustrated in Fig. 15. In each plot, the bottom x-y plane represents the space of neuromuscular parameters—program phase durations, motor neuron firing frequencies, muscle contraction amplitudes, and now also higher-level parameters of inter-muscle coordination—of which only two dimensions are shown for clarity. The black dots represent individual cycles. Plotted in the vertical, z dimension is a hypothetical surface of functional performance that can be evaluated at each point in the parameter space—for instance, the amount of food (seaweed) eaten. Performance is higher for some parameter combinations than for others. Say that acceptable performance occurs above the red contour on the performance surface, and correspondingly within the red circle(s) in the parameter plane.

An important point is that the functional task with respect to which the performance is evaluated remains constant. If the task changes from cycle to cycle, the performance surface will alter, and it is naturally to be expected that variability will thereby be introduced into the set of neuromuscular parameters that the system generates to produce performance on that surface. To avoid this, we, like Horn et al. (2004), have attempted in our experiments here to keep the “task” constant by stimulating the esophageal nerve in a constant, regular manner. What we are trying to understand is the large variability that, even faced with a completely constant stimulus, the system nevertheless continues to generate.

We can now envisage three possibilities. The first is the traditional tight coupling scenario (Scenario 1, left in Fig. 15). Only few parameter combinations produce adequate performance, and the system precisely selects and matches the parameter values so that only those combinations are ever generated. This is what happens, apparently, in the tight coupling between the two ARC muscles. Tight coupling—indeed a precisely identical synchronization and symmetry—between the two sides of the animal may indeed represent one of the real control variables of the system (see Introduction). Note, however, that demonstrating tight coupling between parameters does not yet prove that that coupling is functionally necessary, if, as in this case, the precise shape of the surface of functional performance remains unknown. It could be argued that while the coupling between the two sides of the animal probably plays some positive role, it is established mainly because, with
standard neurophysiological mechanisms, it is relatively easy to establish a precise identity between the activities of two components, as opposed to a different, but still precise relationship such as would be required for tight coupling between the antagonistic ARC and opener muscles.

In any case, there apparently is no such tight coupling between the ARC and opener muscles, and in general—except between the corresponding components on the two sides of the animal—loose coupling appears to be the rule in the system (Fig. 13). Again, a caveat remains. Note that Scenario 1 is drawn to allow the possibility that there might exist multiple, perhaps completely disconnected, peaks of high performance. In other words, there might be multiple solutions to the functional problem—despite the tight coupling, still a degenerate mapping from the neuromuscular parameters to functional performance, or multiple degrees of freedom in accomplishing the functional task. Biomechanically, there is indeed degeneracy in the Aplysia feeding neuromusculature (Brezina and Weiss 2000; Brezina et al. 2000b; Drushel et al. 1997, 1998; Neustadter et al. 2002a,b). In particular, in addition to the ARC and opener muscles, there are other muscles that can close and open the radula in the buccal mass (see, e.g., Morton and Chiel 1993a,b). Because we have not studied all of the muscles of the buccal mass simultaneously, it could still be argued that, say, the contractions of all of the closer muscles complement each other, as do those of the opener muscles, so that, while there is no coupling between any individual pair of closer and opener muscles, there is nevertheless tight coupling between the high-level closer and opener functions. We cannot at present completely rule out this interesting possibility, but we consider it unlikely. In particular, there appears to be a basic argument against Scenario 1, as the general scenario of the functional organization of the Aplysia feeding neuromusculature, in any form (see following text).

Thus rather than being tightly and precisely organized, the neuromuscular parameters must evidently be pictured as being randomly scattered over a large part of the x-y plane in Fig. 15. Nevertheless, adequate performance might still always be assured if the surface of functional performance is very broad (Scenario 2, middle in Fig. 15), if the neuromuscular system has very large tolerances, permitting a highly degenerate mapping from the neuromuscular parameters to functional performance. In other words, tight coupling is absent, but this does not matter because it is not needed for function. This is contrary to what is usually expected in an organized and coordinated neuromuscular system. Even more importantly, the same basic argument can be raised against Scenario 2 as against Scenario 1.

What is common to Scenarios 1 and 2 is that they both assume that, no matter how the neuromusculature operates internally, in each cycle the activities of its various components complement each other sufficiently so that the internal variability never emerges in the functional performance of behavior. Functional performance is always at least adequate, and indeed, with a constant functional task, quite similar and stereotyped, in each cycle. However, there is increasing evidence that this is not the case in Aplysia feeding behavior. Consider Scenario 3 (right) in Fig. 15. Here the surface of functional performance is not overly broad, and, with the large variability of neuromuscular parameters, some cycles of behavior fail to achieve adequate performance. There is already evidence for such unsuccessful, apparently dysfunctional cycles of feeding behavior in Aplysia [see, e.g., Fig. 8 of Horn et al. (2004); Morton and Chiel 1993a]. Furthermore, our preliminary data (C. Lum, Y. Zhurov, V. Brezina, unpublished data) now indicates more generally that even when faced with a constant, regular task, a standard seaweed strip that the animal must swallow, spaghetti-like, over multiple cycles, the movement of the strip into the mouth—that is, the functional performance of the task—is extremely variable from cycle to cycle, and includes, again, cycles with no movement at all. Thus we favor Scenario 3 as the best representation of the functional organization of the neuromusculature in Aplysia feeding behavior.

Of the three scenarios in Fig. 15, Scenario 3 is perhaps the most surprising. In agreement with the conventional view, tight coupling between the neuromuscular components is needed for function. But the system does not constrain the combination of neuromuscular parameters that it generates so as to provide that tight coupling in any particular cycle. Consequently, in some cycles it pays the penalty of inadequate or completely dysfunctional performance.

Comparison with other systems

How unusual is our finding of a lack of tight coupling in the neuromusculature? In the very complex neuromuscular system of a vertebrate, during the performance of an arbitrary voluntary task such as reaching for an object in space or even a behavior such as locomotion that is intrinsically more stereotyped but must respond to a constantly varying environment (see, e.g., Swinnen 2002; Todorov 2004; Todorov and Jordan 2002; Zajac 1993), a study of just two muscles, out of the hundreds involved, might indeed have found a lack of coupling such as we found here. A truer comparison, however, is with other stereotyped behaviors performed with a relatively small neuromusculature—though this may still comprise tens of muscles—under conditions that remain the same from trial to trial. Three well-studied examples of such behavior are the various vertebrate scratching and wiping reflexes (Gisztzer 1995; Kargo and Gisztzer 2000; Poppele and Bosco 2003; Smith and Zernicke 1987; Stein 1995), arm movements in octopus (Gutfreund et al. 1998; Sumbre et al. 2001), and vertebrate swallowing (reviewed by Ertekin and Aydogdu 2003). In each case, there is a well-defined sequence of activation of the muscles, with well-defined relationships between their contractions, that is followed in each repetition of the task. This is tight coupling: Scenario 1 in Fig. 15. In such a case, we would have found a good correlation in our analysis in Fig. 14. If the sequence is disrupted, as happens for instance in human swallowing after stroke (Ertekin and Aydogdu 2003), the behavior fails. And indeed the Aplysia swallowing behavior, as already mentioned, does fail in some cycles. In this respect, the Aplysia behavior differs even from a complex vertebrate behavior such as reaching for an object, where, although the many degrees of freedom may allow a different neuromuscular strategy to be used on each repetition, the behavior succeeds each time. We believe therefore that the lack of tight coupling that we have found in the Aplysia system is not merely the result of incomplete observation of a system that, on some level, is in fact tightly coupled, but, on the contrary, gives us a relatively true picture of the complete system that really is loosely coupled.
Why is the *Aplysia* feeding CPG variable?

Horn et al. (2004) provided evidence that the variability at all levels in the *Aplysia* feeding system is driven by the variability of the motor programs generated by the CPG. In view of the functional penalties that this apparently incurs in some cycles, why does the CPG generate this variability? Our proposal, previously set out in Horn et al. (2004) and Brezina et al. (2005), is that the penalties in individual cycles are outweighed by higher-order benefits of the overall functional arrangement that permits them.

In brief, the variability serves to generate a beneficial diversity of feeding movements. At any point in its meal, the animal may be confronted with any of a wide range of seaweed types and qualities that are best ingested with somewhat different feeding movements. It is likely, furthermore, that the precise requirements of the best movement at any point are unknown. External tactile and chemical cues cannot fully distinguish, for example, the toughness of a piece of seaweed. How the seaweed can best be eaten, indeed whether it can be eaten at all, can only be determined, by internal sensory feedback from the buccal mass and esophagus, once the attempt has actually been made. In these circumstances, a trial-and-error strategy may be best. The CPG randomly generates a wide range of movements that efficiently sample the entire space of likely possibilities. (There is indeed great variability in the movements of the buccal mass during feeding): see Drushel et al. 1997, 1998; Neustadter et al. 2002a,b; Zhurov et al. 2005.) In this light, the identity that is always maintained between the two sides of the animal can be seen as a priori built-in “knowledge” that no asymmetrical movement is ever likely to be a good movement. In contrast, along many other dimensions of the movement such as that governed by the relationship between the ARC and opener systems, diverse movements are worth trying. Some of these movements will fail—and in a herbivore the penalty for failure in any particular cycle is relatively low—but at least some will succeed. Modeling studies suggest that such a strategy can guarantee, on average, the best functional behavior that is always maintained between the two sides of the animal can be seen as a priori built-in “knowledge” that no asymmetrical movement is ever likely to be a good movement.

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