Motor neuron activity is often insufficient to predict motor response

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Our understanding of the necessity of considering peripheral properties when investigating how neural activity generates behavior has significantly increased in recent years. These advances include a theoretical analysis of the neuromuscular transform and a deeper understanding of the functional effects of non-linear contractile responses, slow muscle relaxation, and neuromodulation.

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Abbreviations

5-HT	5-hydroxytryptamine, serotonin
DUM	dorsal unpaired medial
PD	pyloric dilator

Introduction

The neural networks that generate motor neuron activity patterns evolved to create motion. However, the spike trains produced by motor neurons are not motion. Motion instead results from muscles transforming motor neuron activity into movement [1]. This distinction would be unimportant if muscles proportionally followed their input and if the intrinsic properties of muscles were constant. However, work in many systems has shown that the neuron-to-muscle transform is non-linear and subject to modulation. The neural basis of behavior can therefore be fully understood only by considering both neural activity and peripheral properties.

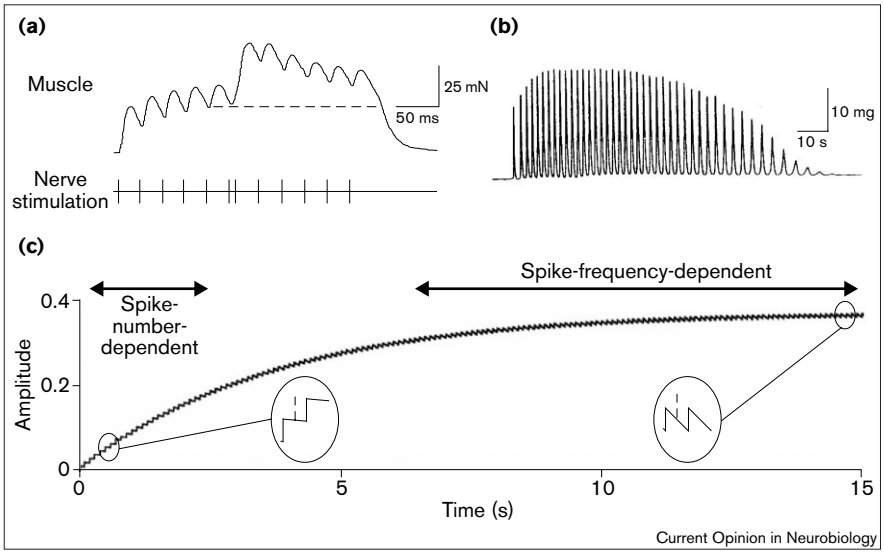
Non-linear contraction properties

Motor neuron spike frequency alone is often insufficient to predict muscle contraction. The primary reasons for this difficulty are catch, active muscle properties, and slow muscle contraction dynamics (Figure 1).

Catch

Catch occurs when the addition of extra spikes to a spike train induces a long-lasting increase in contraction amplitude. In Figure 1a an extra spike was added after the sixth spike in the train; this single spike increased contraction amplitude for the remainder of the train. The muscles in which catch has been observed now include human quadriceps and thenar (thumb) and rat medial gastrocnemius and diaphragm [2–7]. Gorassini *et al.* [8•] have shown that during walking in the rat, neurons innervating fast-twitch leg motor units initiate their bursts with doublet or triplet spikes. Initial doublet or triplet firing induces faster and larger contractions, which may help produce the rapid force onset that is necessary during walking. It thus appears that catch is widespread, and that motor neuron firing is adjusted to take advantage of it.

Figure 1



Non linear contraction properties. **(a)** Catch occurs when the addition of extra spikes to a spike train induces a long-lasting increase (dashed line) in contraction amplitude. In the case shown here, a single spike has been added after the sixth spike in the train. Top trace, muscle contraction (rat medial gastrocnemius); bottom trace, motor nerve stimulation. Modified from [2]. **(b)** Active muscle properties. A muscle (pyloric dilator, PD) from the shrimp (*Palaemon serratus*) stomatogastric nervous system myogenically produces rhythmic contractions in the presence of the neuromodulator YGGFMRFamide. Modified from [9]. **(c)** These model results show why in slow muscles spike number determines contraction amplitude early in a train and spike frequency determines amplitude late in a train. Early in the train, relaxation is very slow, and changing the interspike interval has little effect on the achieved contraction amplitude (the vertical dashed line in the left inset occurs at an interspike interval that is half of the original, but results in nearly the same achieved contraction amplitude). Late in the train, relaxation is rapid, and changing the interspike interval results in large amplitude changes (vertical dashed line in right inset). Amplitude units are arbitrary. Modified from [11].

Active muscle properties

Muscles that can myogenically produce contractions, or that contract in response to stretch, are not uncommon in invertebrate striated muscle, including many motor pattern model systems. Figure 1b shows an example of myogenic rhythmicity from a pyloric muscle of the crustacean stomatogastric system [9]. Some 120 s before the beginning of the trace, a neuromodulator was added to the perfusion. This application induced a ~1 min bout of myogenic muscle contractions. Similar active properties are present in striated muscles in the *Aplysia* feeding system [10•].

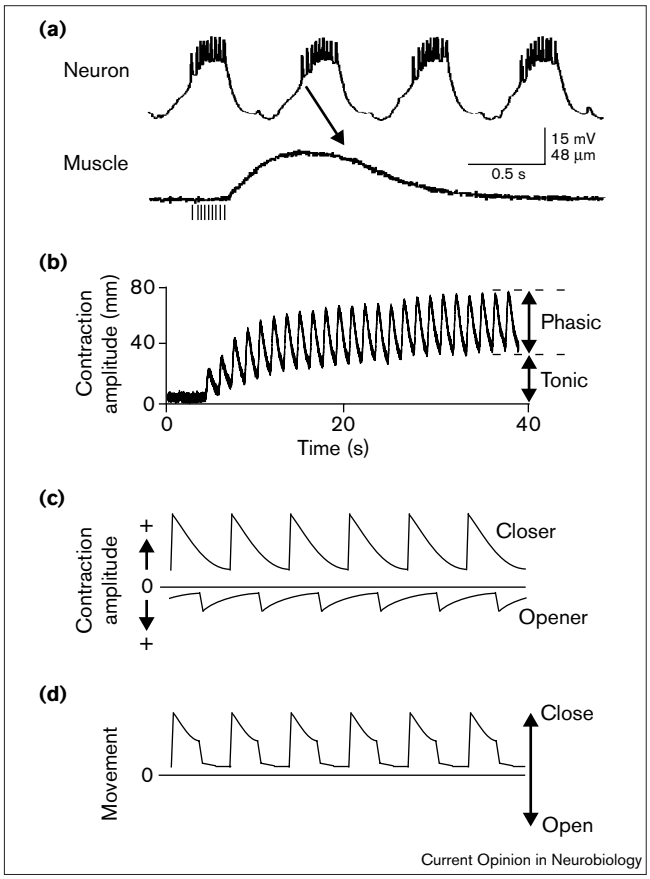
Slow muscle contraction dynamics

Many invertebrate striated muscles show graded responses to neuron input, and single spikes induce only small unitary contractions. In such systems, contraction amplitude depends on spike number early in the train, but on spike frequency late into the train [11]. Figure 1c shows the results of a model of slow muscle that explains why this change occurs. In this example, nerve stimulation starts at the beginning of the trace. Each upward small step is the contraction induced by a single spike, and the decline after each step is the muscle relaxation that occurs between each spike. In this model, muscle relaxation between spikes is an exponential function of muscle amplitude. Thus, when contraction amplitude is small (early in the train), muscle relaxation is extremely slow. Consequently, across a wide range of interspike intervals, changing when the next spike occurs causes only very small changes in the summated contraction amplitude the muscle achieves. For instance, the dashed vertical line in the left inset shows that if the next spike occurs at half the original interspike interval, the amplitude achieved after this early spike is nearly the same as when interspike interval is constant. Therefore, early in the train, spike number, not spike frequency, primarily determines contraction amplitude. As contraction amplitude increases later into the train, since muscle relaxation is exponential, relaxation slope becomes large. At this time, changing interspike interval produces large amplitude changes (dashed vertical line, right inset), and amplitude depends on spike frequency. Between the spike number- and spike frequency-dependent zones, both spike number and frequency determine contraction amplitude.

Muscle relaxation can temporally mismatch neuronal input

Muscles relax slowly, and even fast-twitch fibers can take 150 ms to reach full relaxation. This inability of muscles to follow rapid decreases in neuron firing rates complicates the task of correctly ending rapid movements. The agonist–antagonist–agonist activity pattern characteristic of rapid movements exists in part to solve this problem. During rhythmic movements, slow relaxation can cause another difficulty — temporal summation between muscle contractions [12,13••–15••]. The top trace in Figure 2a shows an intracellular recording from a pyloric motor neuron of the stomatogastric nervous system. This neuron rhythmically fires action potential bursts every 750 ms.

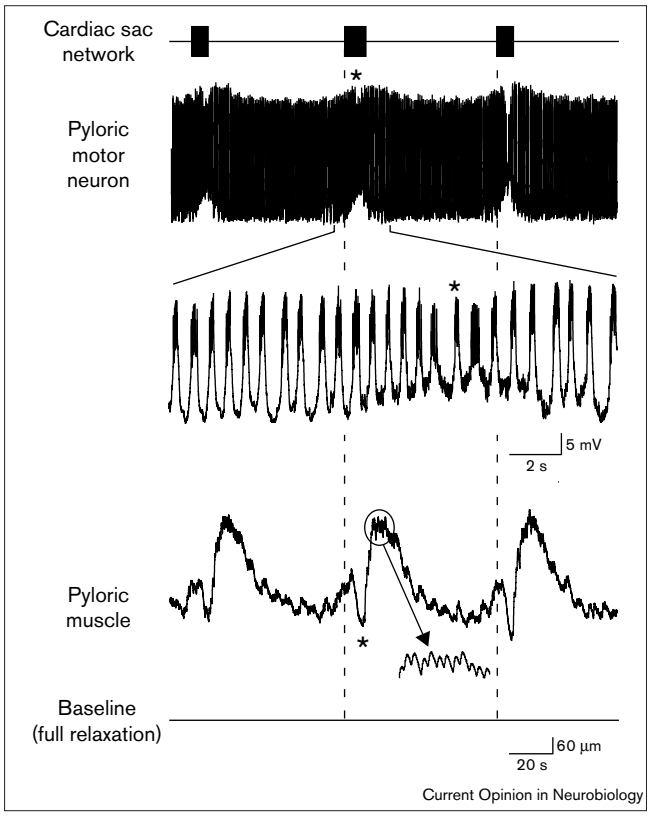
Figure 2



Intercontraction summation can limit the functional range of rhythmic motor pattern production. **(a)** Top trace, intracellular recording from the PD neuron of the lobster stomatogastric system. Bottom trace, isotonic recording of the muscle the neuron innervates. The neuron fires in a bursting pattern with a cycle period of ~750 ms. When the muscle's motor nerve is stimulated to mimic the first neuron burst in the trace, the contraction is very slow; had the nerve been stimulated to mimic the next burst, the second contraction would have begun (arrow; angled in order to account for contraction delay) before the first ended. **(b)** When the motor nerve is stimulated with rhythmic bursts, the contractions summate; at steady state, muscle contraction consists of a sustained tonic contraction on which phasic contractions are superimposed in time with the rhythmic stimulation. The train starts 5 s into the trace; each contraction (upward deflection) corresponds to one burst in the stimulation train. **(c)** Schematic of closer and opener muscle contractions in the *Aplysia* feeding system during rapid feeding. Opener muscle contractions have been flipped vertically – the downward deflections are muscle contractions; the upward curves are relaxations. Horizontal line represents full relaxation; neither muscle ever fully relaxes. **(d)** Algebraic sum of contractions in (c); horizontal line indicates threshold of radula (a feeding organ) opening. In this model, the radula never opens because opener muscle contractions cannot overcome closer muscle tonic contraction, and thus rapid feeding would be impossible. (a,b) modified from [12]; (c,d) modified from [15••].

The lower trace in Figure 2a shows a recording of the contraction induced in this neuron's muscle by motor nerve stimulation (vertical lines under the trace) matching the first neuron burst in the trace. The muscle relaxes very slowly, and in a rhythmic stimulation paradigm, the next

Figure 3



Muscles that relax slowly in comparison to their rhythmic inputs can extract low frequencies from broad-band neuronal input. Top trace, schematic of the activity of a very slow (cycle period ~1 min) lobster stomatogastric network, the cardiac sac network. Second trace, intracellular recording from a PD neuron of the rapid (cycle period ~1 s) pyloric network. Cardiac sac activity alters PD neuron activity, but at all times the PD neuron continues to cycle with a short cycle period (third trace, expanded time scale). Fourth trace, isotonic contraction of the muscle innervated by the PD neuron when the motor nerve is stimulated with the neuron activity in trace 2. The pyloric-timed contractions are very small (inset; each upward deflection is the contraction induced by a single PD neuron burst), and the muscle's motor output is almost exclusively cardiac-sac-network-timed, even though no cardiac sac neuron innervates the muscle. Asterisks mark the same time in traces 2, 3, and 4, and show that the minimum tonic contraction amplitude is associated with a several-cycle-long reduction in pyloric neuron activity. The 20 s time bar applies to all traces except the time expansion diagram (third trace). Modified from [16••].

burst would arrive before the muscle had fully relaxed. Figure 2b shows the muscle's response to stimulation with a rhythmic train of bursts. Initially, each contraction 'builds' on the relaxation phase of the preceding contraction, and thus a sustained, tonic contraction develops. At stable state (where time > ~30 s), muscle contraction consists of a tonic contraction on which phasic contractions are superposed in time with the rhythmic neural input.

Modeling work in the sea slug *Aplysia* suggests that tonic contraction would limit the range of rhythmic motor patterns that animals could produce [15••]. *Aplysia* feeds by means of a hand-like structure called the radula that is extended from the buccal cavity, closes to grasp food, is retracted into the esophagus, opens to release the food, and is then re-extended for the next bite. Models of the opener and closer muscles show that, in the absence of mechanisms that can change muscle contraction properties (see below), during rapid feeding neither muscle would fully relax (Figure 2c). Figure 2d is the algebraic addition of the contractions in Figure 2c, and thus shows the radula movement predicted by the muscle activity in Figure 2c. The radula never opens because the opener muscle contractions cannot overcome the closer muscle tonic contraction, and thus the animal would be unable to produce rapid feeding movements.

Slow muscles can extract low-frequency modulation from their neural input

Morris *et al.* [16••] have shown in the lobster stomatogastric system that slow muscles can extract low-frequency components from broad-band neuronal input. The top trace in Figure 3 shows the activity of a very slow (cycle period ~1 min) stomatogastric neural network, the cardiac sac network. The second trace shows the activity of a motor neuron from the rapid, pyloric network (cycle period ~1 s). Pyloric motor neuron output is modified by cardiac sac activity (as illustrated in more detail in the time-expansion diagram, third trace), but at all times the neuron continues to fire with a short cycle period. The muscle innervated by the pyloric neuron relaxes very slowly in comparison to the pyloric cycle period (the same muscle is shown in Figure 2a), and its primary response to rhythmic neuronal input is tonic contraction. As noted earlier, tonic contraction amplitude results from temporal summation between pyloric motor neuron bursts. Consequently, tonic contraction amplitude varies if neuron cycle period or spike number changes [12]. During each cardiac sac network burst, pyloric neuron activity decreases for several cycles (represented in Figure 3 by the asterisks) and, subsequent to each cardiac sac burst, pyloric neuron activity increases for 10–15 cycles before returning to control levels. Tonic contraction amplitude consequently varies in phase with cardiac sac activity (bottom trace). The amplitude varies by ~50% of the muscle's maximum contraction amplitude, and this muscle thus almost exclusively expresses a cardiac sac motor pattern, even though no cardiac sac motor neuron innervates it.

Neuromodulatory inputs can match muscle and neural input temporal properties

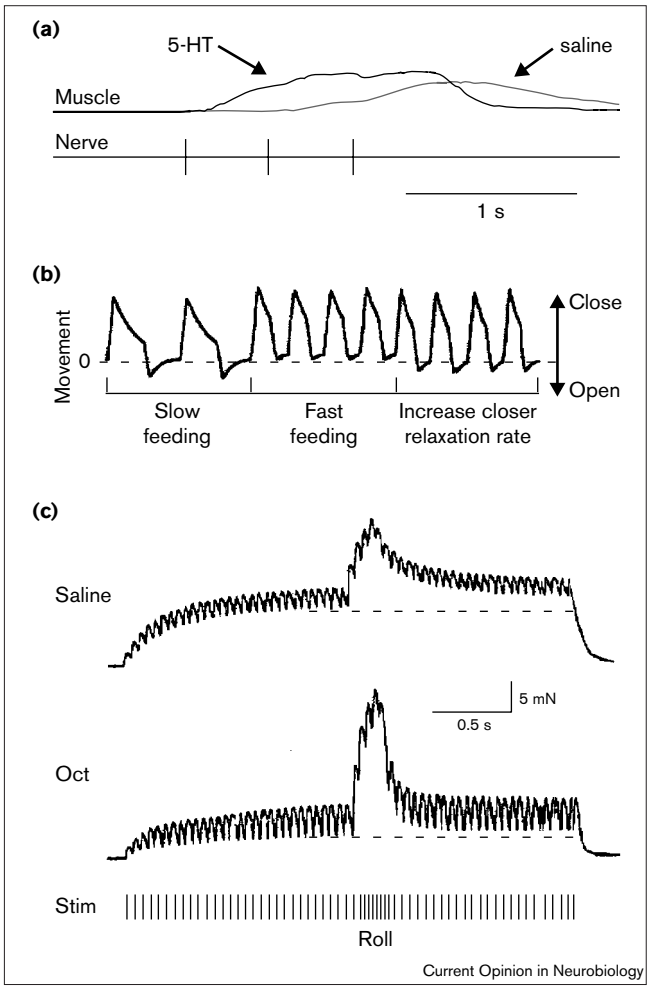
One example of muscle modulation is shown in Figure 1b, in which peptide application induces myogenic rhythmicity in a quiescent muscle. Similar modulation of myogenic rhythmicity is present in *Aplysia* and *Helix* [10•,17]. Modulation of the amplitude as well as of the contraction and relaxation rates of neurally evoked contractions is also widely present in invertebrates, including well-known model systems such as locust, *Aplysia*, and the stomatogastric system (recent references include [10•,18–21,22•,23••]). Figure 4a shows an example of modulation of *Aplysia*

opener muscle contraction [10•]. The top traces show two isotonic muscle contractions, one in saline and the other in 5-HT (5-hydroxytryptamine, or serotonin), in response to identical, three spike neuron firing. This muscle both contracts and relaxes much more quickly in 5-HT.

In *Aplysia*, the functional effects of muscle modulation are beginning to be understood. *Aplysia* radula closer and opener muscles are slow, and intercontraction temporal summation could disrupt rapid feeding (Figure 2d). In addition to the transmitters that induce muscle contraction, the motor neurons innervating both muscles contain neuromodulatory transmitters that increase relaxation rate (which decreases temporal summation), and these neuromodulators would be preferentially released during rapid feeding [23••]. Figure 4b shows that increasing closer relaxation rate alone would restore function during rapid feeding. Increases in opener contraction and relaxation rate would further facilitate feeding by decreasing antagonist muscle co-contraction.

Similar advances in understanding the function of muscle modulation have been made in the octopaminergic dorsal unpaired medial (DUM) neuron system in insects. This system was once thought to be a collectively activated arousal system, but recent work shows that the DUM neurons can be subdivided on the basis of differential synaptic input and activity [24,25•–28•]. Particularly relevant observations concern DUM input to locust wing and leg muscles. The wing muscle is anatomically appropriate for generating lift and for causing flight rolls (rotations around the anterior–posterior body axis). However, in saline the muscle develops a sustained tonic contraction when stimulated at flight frequency, and stimulation mimicking a roll induces a long-lasting tonic contraction increase (Figure 4c). Tonic muscle contraction is inappropriate for rhythmic behaviors such as flight and flight corrections, and the function of the muscle has therefore been uncertain. The muscle receives DUM neuron innervation, and recent work [18] has shown that octopamine greatly reduces its tonic contraction and long-lasting response to roll-mimicking stimulation (second trace, Figure 4c), thereby providing a mechanism for behaviorally appropriate contraction. Similarly, walking activates DUM neurons that innervate leg muscles, and again octopamine decreases tonic muscle contraction [29]. Comparable results have been obtained in *Manduca* [30•]. The DUM modulatory system thus may function, in part, to match muscle properties to central activity.

Figure 4



Modulation can match neural input and muscle temporal properties. **(a)** Isotonic recordings of *Aplysia* opener muscle contractions in saline (gray) and 5-HT (black) in response to identical, three spike, motor neuron firings. In the presence of 5-HT, muscle contraction and relaxation rates are much increased. **(b)** Muscle modulation can restore feeding. Radula movements during slow feeding (left), fast feeding (middle), and fast feeding with increased closer muscle relaxation rate (right). When feeding speed increases, feeding is disrupted because of closer muscle temporal summation (see Figure 2c for muscle contraction profiles); increasing closer muscle relaxation rate decreases temporal summation and restores feeding. **(c)** Upper trace, locust flight steering muscle activity, induced by the motor nerve stimulation (Stim) shown in the bottom trace, in saline. The muscle develops a large tonic contraction, and stimulation mimicking a flight roll induces a long-lasting increase in tonic contraction (dashed line). The lower trace shows the activity of the same muscle, in response to the same motor nerve stimulation, in octopamine (Oct). The tonic component, and the duration of the increased tonic contraction associated with the roll, is much reduced. (a) modified from [10•]; (b) modified from [23••]; (c) modified from [18].

A general theoretical framework for analyzing the neuromuscular transform

Although investigators typically describe the neuron-to-motor transform specific to the system that they are studying, a general treatment of neuromuscular transformation has been previously unavailable. This void has been filled by a landmark series of papers [13••–15••]; these papers provide a theoretical basis for quantitative analysis of neuromuscular transforms, describe how this analysis can be applied to rhythmic motor patterns, and show how modulation of the neuromuscular transform can optimize rhythmic behaviors [13••–15••]. The analysis in these papers is generally applicable, and provides a fundamental conceptual framework for investigating the role of the periphery in behavior.

Broader implications

Most of the data described thus far have been collected from invertebrate studies. However, several considerations suggest that the issues raised above may also apply to vertebrate nervous and neuromuscular systems.

Rapid skeletal movements in vertebrates

Early studies on muscles, and recent work [31] on single motor units, suggest that intercontraction temporal summation in vertebrate skeletal muscles should occur during rhythmic movements with cycle periods of <500–750 ms; intercontraction summation would therefore be expected in rapid motor patterns such as trills in birdsong and music. Unfortunately, we have been unable to find vertebrate references dealing with the production of such rapid rhythmic motor patterns. It is, however, intriguing that plasma levels of epinephrine, which increases slow muscle relaxation rate, dramatically increase during running [32,33]. The function of this modulation is unknown but suggests that, in vertebrate skeletal muscle, modulation could be used to match muscle properties to neural input. Extremely slow striated muscles (comparable to those in Figure 2a,b) that receive rapid rhythmic input are present in lower vertebrates [34,35] and, in these preparations, spike number dependence and intercontraction temporal summation could easily be present.

Smooth muscles in vertebrates

Vertebrate smooth muscle is active, slow, graded, and receives extensive modulatory input. Autonomic neural activity is often rhythmic, and this rhythmicity is present in single sympathetic neurons [36,37•–39•]. The relationship between neural and effector activity is not well understood in the autonomic system, but an intriguing speculation is that the same complex interplay between neuronal output and muscle properties observed in invertebrates may also occur in vertebrate smooth muscle.

Neuron membrane and intracellular messenger responses

The kinetics of membrane conductance activation and inactivation, second messenger accumulation and destruction, and protein phosphorylation and enzyme activation can all be slow. Consequently, these processes could show spike number dependency, interburst temporal summation, and low-frequency extraction from broad-band input. Ca^{2+}/calmodulin kinase II levels show temporal summation in response to rhythmic stimulation with parameters appropriate for bursting activity [40,41]. Liu *et al.* [42] have shown that the inclusion of slow calcium sensors in model neurons allows different neural activity patterns to be distinguished. Buchman *et al.* (*Soc Neurosci Abstr* 2000, 26: 2000) have shown that conductances with extremely slow activation kinetics are necessary in order to model the temporal pattern dependency [43] displayed by some pyloric network neurons. Brezina *et al.* [44] have shown that the dependence of peptide release on the pattern of neuron activity can be used to distinguish between different models of the release process. Muschol and Salzberg [45] have shown that 'residual' (equivalent to the 'tonic' contraction component mentioned above) calcium concentration increases during certain patterns of neurohypophysis stimulation, and that release of arginine vasopressin from the neurohypophysis depends in part on this concentration. Liljelund *et al.* [46] have shown clear examples of temporal summation of calcium concentration (analogous to the temporal summation of contraction amplitude shown in Figure 2b) in spontaneous and driven bursting activity in early postnatal Purkinje neurons. Investigation of slow neuromuscular transforms may thus also have relevance to neuronal responses.

Two cautionary examples

Recent modeling work identifies two cases in which the role of central processing may have been misinterpreted as a result of ignoring the periphery [47,48,49•]. The first of these articles [47] shows that intrinsic muscle properties can smooth neuronal firing irregularities so as to produce smooth movements, and thus central neural controllers to achieve this goal are unnecessary. The final two papers [48,49•] suggest that evidence arguing that motor cortex is a high-level controller is an epiphenomenon, and that if the properties of the motor periphery are included, all experimental data are consistent with the older concept that motor cortex is a low-level controller of specific muscle groups. If the predictions of these models are confirmed by experimental data, this work will overturn long-standing beliefs of how neural activity controls motor output.

Conclusions

We have presented here data showing that in many cases the motor output generated by neural activity cannot be predicted without a detailed understanding of the periphery. This difficulty arises from several sources, including: first, the non-linear neural input to muscle contraction transform functions such as catch and endogenous myogenicity; second, the slow (relative to their neuronal input) contraction and relaxation properties of many muscles, which can lead to spike number dependence, intercontraction temporal summation, and low-frequency filtering of broad-band input; and third, the state dependence of muscle response properties introduced by neuromodulation. This work underscores the fundamental role of the periphery in behavior, and the necessity of considering peripheral properties when investigating the way in which organisms create behavior.

Acknowledgements

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This study shows that, when peripheral properties are taken into account, all data suggesting that motor cortex functions as a high-level movement controller can be equally well explained using the older concept that motor cortex is a low-level controller of individual muscle groups.