Electromyography and the evolution of motor control: limitations and insights

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Synopsis Electromyography (EMG), or the study of muscle activation patterns, has long been used to infer central nervous system (CNS) control of the musculoskeletal system and the evolution of that control. As the activation of the muscles at the level of the periphery is a reflection of the interaction of descending influences and local reflex control, EMG is an important tool in integrated investigations of the evolution of coordination in complex, musculoskeletal systems. Yet, the use of EMG as a tool to understand the evolution of motor control has its limitations. We here review the potential limitations and opportunities of the use of EMG in studying the evolution of motor control in vertebrates and provide original previously unpublished data to illustrate this. The relative timing of activation of a set of muscles can be used to evaluate CNS coordination of the components in a musculoskeletal system. Studies of relative timing reveal task-dependent variability in the recruitment of different populations of muscle fibers (i.e., different fiber types) within a single muscle, and left–right asymmetries in activation that need to be taken into account in comparative studies. The magnitude of muscle recruitment is strongly influenced by the instantaneous demands imposed on the system, and is likely determined by local reflex-control systems. Consequently, using EMG to make meaningful inferences about evolutionary changes in musculoskeletal control requires comparisons across *similar functional tasks*. Moreover, our data show that inferences about the evolution of motor control are limited in their explanatory power without proper insights into the kinematics and dynamics of a system.

Introduction

Electromyography (EMG), or the study of activity patterns of muscles, is a relatively old technique and has been used extensively as a physiological and clinical research tool since the early 1900s (see Ashley-Ross and Gillis 2002 for an overview). However, the use of EMG as a tool to gain insight into motor control and its evolution has been restricted to the past four decades. EMG has been an important tool in the study of motor control as it provides insights into how the muscles are controlled by the nervous system as evidenced by the coordination of muscles in a given movement (Loeb and Gans 1986). In this context, EMG has been used extensively to try to understand motor-control strategies used by the central nervous system (CNS), whether it be from higher control centers, or local reflex arcs, and to understand the functional role of muscles in generating movements (Loeb and Gans 1986).

Movements are remarkably diverse as are the underlying bones and muscles that are responsible for generating those movements. Moreover, although some movements can be understood as relatively simple lever systems, others are the result of the coordination of many monoarticular and multiarticular muscles. Understanding how muscles are controlled to generate the movements, and understanding how the control changes as the underlying morphology changes has been of interest to functional morphologists. Given the apparent complexity associated with the control of multi-jointed musculoskeletal systems, previous authors have suggested that the central control is highly conserved in evolution despite the obvious

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changes in the musculoskeletal systems across taxa (Bramble and Wake 1985; Alfaro and Herrel 2001; Wainwright 2002). More recently, however, an important role for the intrinsic mechanics in simplifying control has been advocated (Aerts et al. 2001; Van der Leeuw et al. 2001; Nishikawa et al. 2007).

EMG has often been used as a tool to gain insights into these issues as it provides a relatively direct representation of how the muscles are activated by the brainstem, spinal cord, and higher control centers. By comparing which muscles are activated, and how these muscles are activated (i.e., the sequence, duration, and magnitude of activation) in order to execute a given movement or functional task, we can gain insights into basic neuromuscular control. The complexity in doing so lies in deciding which aspects to study and how to treat them so as to make meaningful comparisons across individuals and species; the intrinsic variability of the activation patterns provided the greatest obstacle.

Here, we use examples from recently collected, as well as previously published electromyographic data on feeding and locomotory systems in vertebrates, to illustrate a number of potential pitfalls and shortcomings of using electromyographic data in a comparative context. Given these limitations, however, we do believe meaningful insights can be gained from the interpretation of muscle activation patterns. Finally, we discuss the importance of the dynamics of any musculoskeletal system in understanding its control, and we illustrate this using examples from recent and previously published work.

Materials and methods

Animals

Lizards

Three adult male *Pogona vitticeps* (Bearded dragon) were used in the feeding experiments. Animals were housed individually in a climatically controlled room set at 24°C and were provided with a basking spot of 50°C. Lizards were fed with vegetables, crickets, mealworms, waxworms, superworms, and newborn mice twice weekly. Water was always available to the animals. Two additional adults, housed at the animal care and use facility of Northern Arizona University and maintained under similar conditions, were used in the experiments on muscle physiology.

Frogs

One adult *Phyllomedusa bicolor* (Giant leaf frog) and three adult *Litoria caerulea* (White's treefrog) were used in the experiments on locomotion. The animals were maintained in a glass terrarium with dense vegetation and high relative humidity. The temperature fluctuated between 26°C (day) and 20°C (night). A large reservoir of water was present in the terrarium and the animals were fed with waxworms and large grasshoppers *ad libitum*.

Electomyographic recordings in *P. vitticeps*

Small metal markers were inserted on the right side or along the medial plane at the anterior tip of the upper and lower jaw, at the posterior end of the tooth row on the upper and lower jaw, at the base and top of the quadrate, at the anterior and posterior aspect of the parietal and frontal bones, in the neck at the level of cervical vertebrae 2 and 6, in the anterior and posterior regions of the tongue,and at the intersection of the basihyal and hypohyal. Animals were filmed in lateral view while feeding on waxworms, mealworms, superworms, small and large crickets, and grasshoppers using Redlake MotionPro2000 set at 250 Hz and attached to a Philips image intensifier. X-rays were generated using a Philips optimus M200 X-ray generator at 50 KV.

Four bipolar stainless steel twisted-hook electrodes were inserted percutaneously into both the left and right *m. adductor mandibulae externus medialis*. During implantation of the markers and electrodes, animals were anesthetized using ketamine (200 mg/kg body mass). The first electrode on each side was inserted starting at the anteriormost border and electrodes were equally spaced from there to the posteriormost border of the muscle. Additionally, a bipolar Ni-Cr twisted-hook electrode was inserted percutaneously into the left and right *m. depressor mandibulae*. Signals were amplified 10,000 times using Gould Universal preamplifiers with notch filter and Honeywell Accudata 117DC amplifiers. Signals were recorded digitally on tape using a TEAC 145T DAT recorder. To allow synchronization between the X-ray video recordings and muscle activity patterns, a synchronization signal from the X-ray generator was recorded on tape. Data were transferred digitally to a PC using the TEAC QuickVu software and were quantified in Microsoft Excel.

X-ray recordings and EMG in *P. bicolor* and *L. caerulea*

Small metal markers were inserted subcutaneously at the proximal and distal ends of the humerus, at the proximal and distal ends of the radius, at the base of the carpals, at the base of the phalanges, and at the last phalanx of digit II. During implantation of radio-opaque markers, animals were anesthetized using a buffered MS222 solution. Upon recovery, animals were filmed in lateral view while moving on a narrow dowel (17 mm). X-rays were generated using a Philips

optimus M200 X-ray generator and recorded at 200 Hz using a Philips image intensifier attached to a Redlake MotionPro2000 camera.

Bipolar Ni-Cr twisted-hook electrodes were inserted percutaneously into the *m. palmaris profundus* and the *m. flexor digitorum communis longus* in *P. bicolor*, and only into the latter muscle in *L. caerulea*. Signals were amplified 10 000 times using Gould Universal pre-amplifiers with notch filter and Honeywell Accudata 117DC amplifiers. Signals were recorded digitally on tape using a TEAC 145T DAT recorder. To allow synchronization between the X-ray video recordings and muscle activity patterns, a synchronization signal from the X-ray generator was recorded on tape. Data were transferred digitally to a PC using TEAC QuickVu software, and the onset and duration of muscular activity relative to substrate contact was quantified in Microsoft Excel.

Physiological properties of the *m. sternohyoideus* in *P. vitticeps*

The length/tension properties of the hyoid retractor muscle were investigated in two live, anaesthetized adult *P. vitticeps* (snout-to-vent length 99.07 mm and 107.85 mm). In this experiment, the animals were deeply anaesthetized with ketamine (200 mg/kg body mass), and bipolar stainless-steel electrodes were implanted bilaterally into the hyoid retractor muscle (*m. sternohyoideus*). The animals were kept under deep anesthesia by administering additional ketamine (half the original dose) every 2–3 h. In the experiments, the animal was mounted upside-down in a purpose-built holder, the hyoid was sutured to a muscle lever (Cambridge Technology model 6650 force lever connected to an Aurora Scientific Series 305B lever system controller). Initially, the muscle was twitch-stimulated (Grass S48 stimulator connected to a Grass SIU5 stimulus isolation unit), and stimulation voltage was increased until maximal force output was obtained (at 12 V). In all subsequent recordings, muscles were stimulated at 15 V to ensure maximal muscle recruitment.

Next, the muscle was kept at resting length and stimulated with tetanic trains of 300 ms (2 ms pulse duration) of increasing frequency. The fusion frequency (60 Hz) and tension at fusion were determined. Subsequently, muscle length was varied and the passive tension recorded; the muscle was then stimulated with 300 ms tetanic trains at 60 Hz and the active tension recorded. Throughout the experiment, the temperature of the animal was kept at 32°C by a heat lamp and continuously monitored with a YSI telethermometer and thermocouple. After the recordings, the animals were killed by injection of a lethal dose of ketamine (twice the anesthetic dose). "Resting length" of the muscle was defined as its length when the hyoid was lying at rest in the mouth. Extensions of the muscle, thus involve stretching of the muscle beyond this length and correspond to protraction of the hyoid as observed during capture and transport of prey *in vivo* (Herrel et al. 1995, 1997a).

Stimulation experiment—*P. bicolor* and *L. caerulea*

Bipolar twisted Ni-Cr electrodes were inserted in the *m. flexor digitorum communis longus* in both species and also in the *m. palmaris profundus* in *P. bicolor*. Stimulations were performed on one *P. bicolor* and two *L. caerulea*. The animals were brought under deep anesthesia using Ketamine (225 mg/kg body mass) and the muscles of the right forelimb were exposed. Electrodes were inserted in the middle of the respective muscle bellies and connected to a stimulator (Grass S48). The stimulation circuit was charge balanced by a coupling capacitor and bleed resistor (Loeb and Gans 1986) to avoid muscle damage and undue fatigue. Muscles were stimulated at 12 V with a pulse train of 500 ms at 70Hz, and three ms pulse duration. Animals were positioned on their back on a custom-made platform and the lower arm was immobilized to allow visualization of movements at the wrist and hand. Animals were filmed in combined ventral and lateral view by using a mirror positioned at an angle of 45° to the horizontal at the level of the arm. Muscles were stimulated one by one and movements were recorded. Next, combined stimulations were performed to understand the consequences of coactivation of the different muscles.

All experiments were approved by the animal ethics committee at the University of Antwerp and by the IACUC at Northern Arizona University.

Results and discussion

Electrode placement

Careful placement of electrodes and verification of their positions are important in order to accurately categorize and explain inter-electrode variance in EMG activity. Heterogeneity in timing of activity in different parts of "single" muscles (e.g., masseter or *temporalis*) is well documented in mammals and is of fundamental importance for generating the transverse movements characteristic of mammalian mastication (Hylander et al. 1987; Weijs 1994). In primates, for example, the masseter has two distinct functional components: superficial and deep. On the chewing (or working) side in anthropoid primates (monkeys), the deep masseter shows onset and peak activity before

the superficial masseter does, whereas on the non-chewing (balancing) side, the deep masseter is active after the superficial masseter (Hylander and Johnson 1994; Hylander et al. 2000). The kinematic consequence of these activity patterns is that it is the balancing-side deep masseter that pulls the teeth medially through the late part of the power stroke. This late activity in the balancing-side deep masseter also has consequences for mandibular loading; in combination with laterally directed reaction forces on the working-side teeth, deep masseter ''wishbones'' the mandible, stressing the symphysis, thereby necessitating fusion of the symphysis to strengthen the joint (Hylander 1984, 1985). Significantly, late balancing-side deep masseter activity is also associated with symphyseal fusion in alpacas and horses, whereas earlier cessation of balancing-side deep masseter is associated with an unfused symphysis in goats (Williams et al. 2007). Without careful placement of electrodes matched with prior dissection of conspecific individuals, these important aspects of the function of mammalian chewing muscle would not have been revealed.

In many mammals, heterogeneity in timing of different parts of the *temporalis* are responsible for rotating the mandible about a vertical axis during chewing. The posteriorly directed working-side posterior *temporalis* (PT) fires first, rotating the mandible over to the working side, whereas the balancing-side PT fires last, helping to drive the mandible transversely during the power stroke (Weijs 1994). Anteroposterior heterogeneity in muscle EMG is also found in some lizards, but is not associated with transverse movements of the mandible. Figure 1 shows the results of an experiment when multiple electrodes were inserted from anterior to posterior into a single muscle of a lizard, *P. vitticeps*. Whereas the more posteriorly situated electrodes only picked up a signal during ipsilateral bites, the anteriormost electrode picked up a signal during both ipsilateral and contralateral bites when chewing on soft foods (Fig. 1). These results were highly consistent across individuals and are likely associated with the presence of populations of different fiber types within the muscle, with the anteriormost compartment containing slower fiber types (Throckmorton and Saubert 1982; Herrel et al. 1999b). Indeed, previous authors have demonstrated that many of the jaw adductors in lizards consist of regions with slow fibers surrounded by an area consisting mostly of faster fiber types. Similar results have been observed in primates. Baboon superficial anterior *temporalis* (SAT) and PT muscles have more fast twitch fibers than do the deep anterior *temporalis* (DAT). SAT and PT also are relatively inactive during chewing of soft foods and exhibit faster times for rise and fall than does the DAT (Wall et al. 2005, 2006).

The differences in recruitment pattern of the different regions in these muscles may be a reflection of Henneman's size principle (Henneman 1968). Slower fibers will depolarize first because of their lower threshold, and hence are recruited sooner (Milner-Brown et al. 1973). Recruiting only areas with slow fibers for contralateral bites on soft food makes intuitive sense as high forces are not needed to crush such prey. Moreover, slow fibers are oxidative and can be recruited longer or more often without experiencing fatigue. Similar reasoning may be used to explain differences in muscle recruitment during transport of prey. As the food item gets reduced and transported towards the back of the oral cavity, more and more of the jaw adductors become silent (Herrel et al. 1997a, 1999a). Presumably, only those muscles with a high proportion of slow oxidative (SO) fibers are recruited towards the end of the transport stage (but see Herrel et al. 1999b).

Location-specific recruitment of different parts of the muscle with different populations of types of fibers is not unique to jaw muscles. Recent studies have reported similar site-specific recruitment for locomotor muscles (Hodson-Tole and Wakeling 2007; Higham et al. 2008). Differences in the recruitment of different components of the muscle may have major consequences on the mechanics of musculoskeletal systems (Higham et al. 2008). Moreover, caution should be exerted in comparing motor patterns across individuals, species, or higher-level taxa, as apparent differences in strategies of motor control may just be the reflection of differences in electrode position, or the population of fiber type being recorded.

Left–right asymmetry

Figure 2 illustrates the activity of the medial jaw adductor in *P. vitticeps* while chewing and transporting a waxworm. Note how the jaw adductors are not activated, or are only marginally so, when the prey is positioned on the contralateral side. Thus, in contrast to what was previously thought (Bramble and Wake 1985; Herrel et al. 2001), the recruitment of muscles in bilaterally symmetrical systems need not be synchronous, as demonstrated here by data from *P. vitticeps*. Depending on the type of food that is being chewed, both in *P. vitticeps* and in *Tupinambis merrianae* (Ross and Herrel unpublished), the muscles on the contralateral side may remain silent. In both species, however, harder foods are associated with more balancing-side recruitment of the jaw adductors as has been demonstrated previously for some mammals

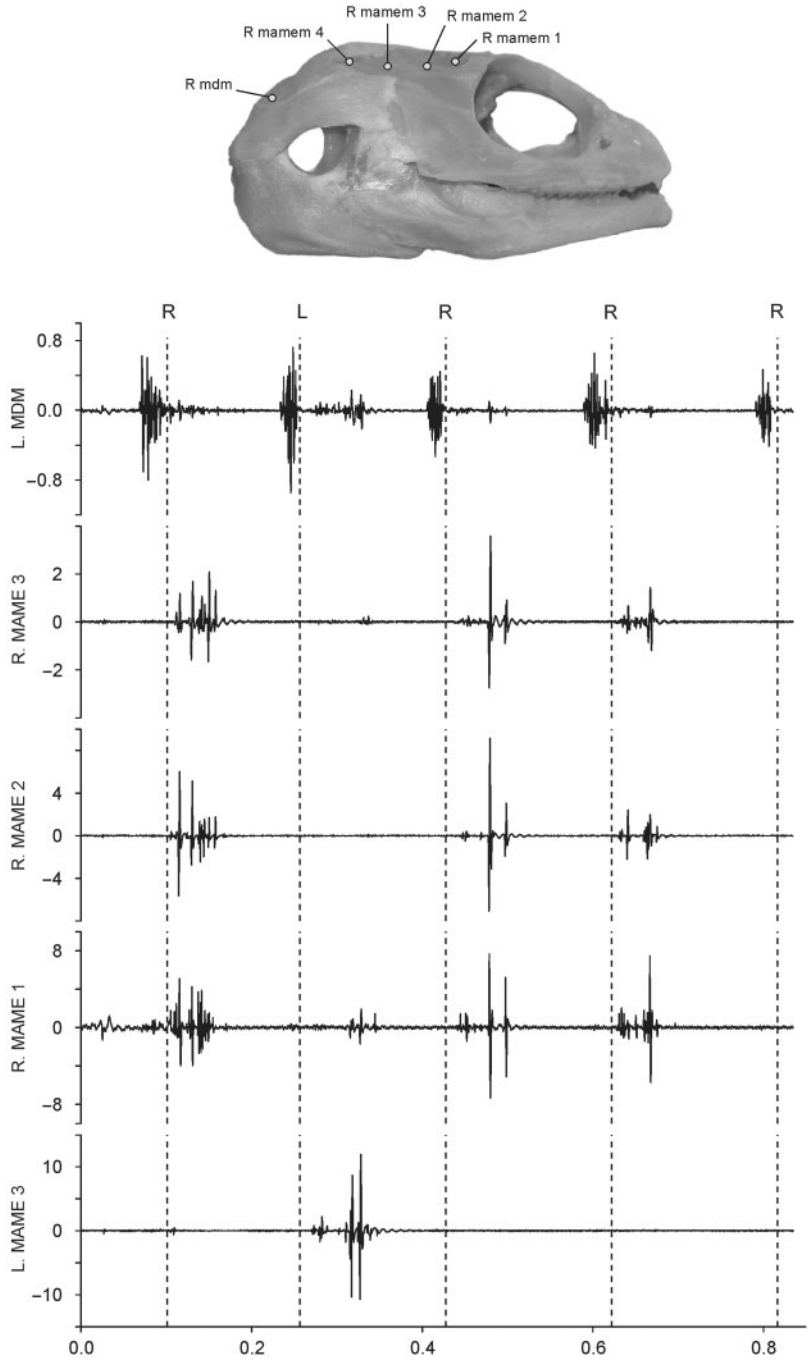

Fig. 1 Representative original traces of the activity of the jaw opener (mdm) and the *m. adductor mandibulae externus medialis* (mamem) during prey transport in *P. vitticeps*. Four electrodes were inserted from anterior to posterior in the same muscle (see top). Note that different parts of the same muscle show distinct activity patterns. Whereas the more posterior portions of the muscle (mamem 2 and 3) are only active during ipsilateral bites, the anterior mamem 1 is also activated during contralateral bites (L, left; R, right).

(Hylander et al. 1992, 2000). Presumably, animals are trying to increase the total force imparted to the food by recruiting balancing-side muscles. For soft prey, this is not necessary and thus only muscles on the ipsilateral side are recruited.

The fact that most previous studies of jaw adductor activity in lizards were performed on animals feeding on relatively large, hard prey (Gans et al. 1985; Herrel et al. 1997a, 1999a) may explain why symmetrical activation of the jaw adductors was often observed (but see Smith 1982). Our data on left–right asymmetry in the activation of the jaw muscles in lizards demonstrate that it is crucial to investigate both sides of a symmetrical system, or at

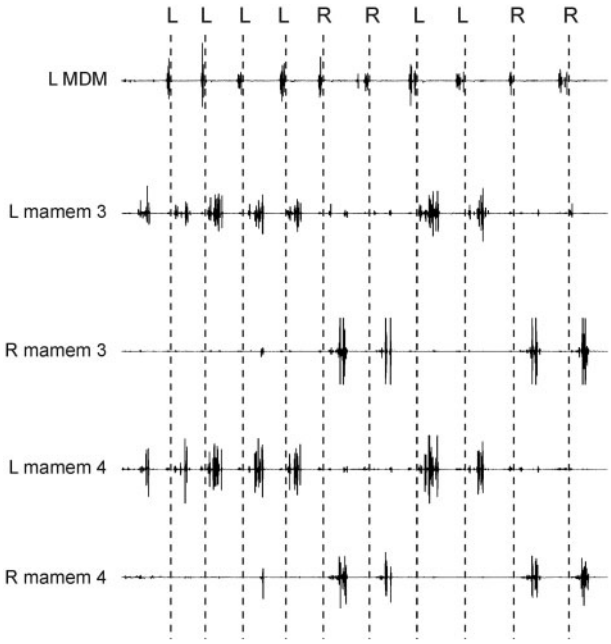

Fig. 2 Representative original traces of the activity of the jaw opener (mdm) and the *m. adductor mandibulae externus medialis* (mamem) during intraoral transport of a waxworm in *P. vitticeps*. Note the very strong differences in recruitment levels for left and right sides of the same muscle depending on the side on which the animal is biting. On top, the bite side is indicated (L, left; R, right).

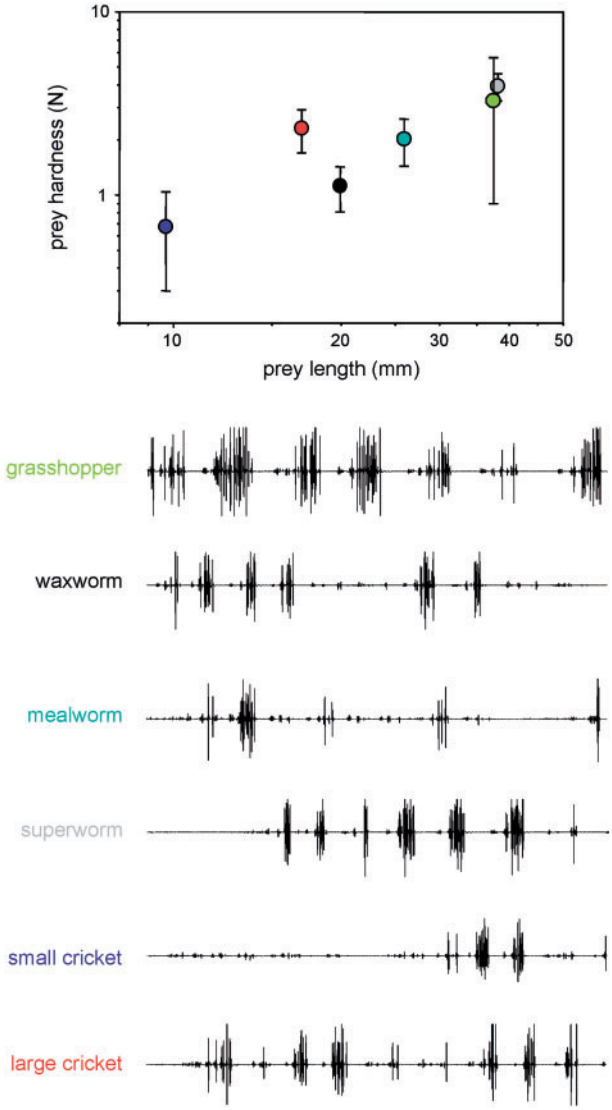

Fig. 3 Top: graph illustrating the relationship between hardness and length of prey for six food items used in the experiment. Note that larger food items are typically harder and that some types are harder than others for a given size. Note the $\log_{10}$ scale on both axes. Bottom: representative original traces of the activity of the *m. adductor mandibulae externus medialis* in *P. vitticeps* while eating different prey items. Traces are derived from a single recording session with identical electrode placement and plotted on the same time and voltage scales. Note how the jaw adductor is more strongly recruited when biting on larger and harder prey types.

least compare similar functional tasks (i.e., balancing-side versus working-side activity) if one wants to compare patterns of muscle recruitment across individuals or species. Asymmetrical activation of cranial muscles may, in general, be more common than previously thought and may even be a primitive feature of vertebrates as unilateral activation has been demonstrated in salamanders (Cundall et al. 1987) and more recently in elasmobranchs (Gerry et al. this volume).

Modulation

In the feeding system, modulation of jaw adductor activity to changing mechanical demands imposed by the structure of the prey can be observed between and within sequences of chewing. Figure 3 illustrates the activation of the *m. adductor mandibulae externus 3* in *P. vitticeps* in response to different prey that were presented. The jaw adductor is recruited for a longer duration, more intensely, and at lower frequency as prey get harder and bigger (e.g., compare grasshoppers with waxworms). Moreover, for softer and smaller prey, the jaw adductors are only recruited during ipsilateral bites, whereas for large and hard prey (e.g., compare the activity for small and large crickets, or for mealworm and superworm) the adductor is recruited on every single bite. Modulation of muscle activation to the mechanical demands placed on the musculoskeletal system is widespread among vertebrates (e.g., Gorniak and Gans 1980; Weijs and Dantuma 1981; Yamada and Yamamura 1996; Herrel et al. 1997b, 1997b; Agrawal et al. 1998; Wainwright and Friel 2000; Kakizaki et al. 2002) requiring careful control of the material properties of food during EMG analyses of feeding. Methods for measuring properties

of food in the field and laboratory are available and should be more widely used (Lucas et al. 2001; Williams et al. 2005). It is of interest that the intensity of muscle recruitment (reflected in the amplitude of the electromyographic signal) seems especially prone to variation as amplitude depends on the instantaneous demands imposed on the system and may largely be governed by proprioceptive feedback loops (Ross et al. 2007). The distribution and functioning of these feedback loops among vertebrates are poorly surveyed. In mammals the proprioceptors in the periodontal ligament act together with the muscle spindles to modulate EMG activity in response to variation in the material properties of food (Hidaka et al. 1997, 1999). Nontetrapods, however, are said to lack muscle spindles altogether, and mammals (and possibly birds) are the only vertebrates shown to have gamma motoneurons that modulate the response properties of the spindles. The implications of these differences for the evolution of vertebrate neuromechanics have only begun to be explored (e.g., Houk 1972; Ross et al. 2007).

One additional issue with using the intensity of muscle recruitment in comparative studies is that the amplitude of the signal is highly dependent on the number of motor units being recorded, and that in turn is dependent on the position of the electrode within the muscle. Even if electrodes are positioned in compartments with similar fiber types, their distance to the motor endplate is likely different and, thus, so will be the amplitude of the signal recorded (Loeb and Gans 1986). Standardization of the amplitude of muscle recruitment relative to a "maximum" recruitment for a given electrode position during a given recording session may alleviate some of these issues. Additionally, time-averaging (i.e., binning) the amplitude component of the signal may take out some of the intrinsic variability in the spike patterns (Larson and Stern 2007). As the coordination of different muscles and temporal components of muscle activation appear less variable, these have often been used by researchers in comparative studies (see Wainwright 2002 for an overview). Temporal aspects of the pattern of muscle activation are, however, still affected by left–right asymmetries in activation and the potential for differences of electrode position with respect to variation in fiber types in a muscle.

Importance of dynamics and muscle mechanics

Interpretation of EMG activity patterns for the function of musculoskeletal control is crucially dependent on the mechanical context of the muscle activity. To illustrate this, we provide two examples that show how an understanding of the context of the patterns of muscle activation can be crucial in interpretation.

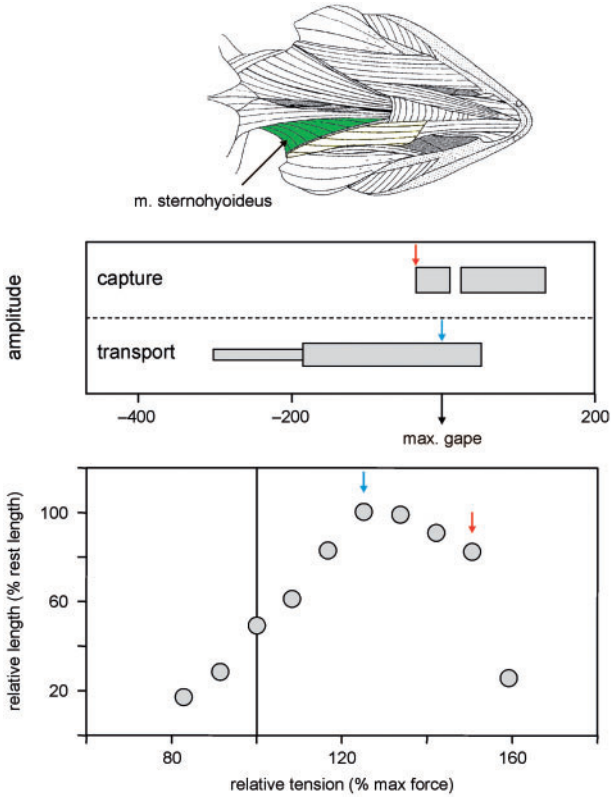

Fig. 4 Top: schematic drawing illustrating the hyobranchial musculature in a typical agamid lizard (*Laudakia stellio*). The hyoid retractor (*m. sternohyoideus*) is colored green. Middle: summary bar graph illustrating the activation of the *m. sternohyoideus* in *L. stellio* capture and transport of a grasshopper. Note the strong difference in the onset and the duration of the activity for the two behaviors. Time 0 = maximal gape. The colored arrows indicate where the hyoid is maximally protracted and the corresponding degree of muscle stretch. Bottom: length-tension diagram for the *m. sternohyoideus* of *P. vitticeps*. Note that the muscle can generate only about 50% of its maximal force when activated at its resting length (vertical line). Maximal output of force is only achieved when the muscle is activated after having been stretched to 30% above its resting length.

Figure 4 illustrates a series of experiments performed to gain understanding of the control of the hyoid retractors in agamid lizards. Agamids typically use their tongue to capture prey. During capture, the tongue is protruded beyond the margin of the lower jaw (Schwenk and Throckmorton 1989; Smith 1988; Schwenk 2000). The hyoid, supporting the tongue, is also protracted during prey capture to a position where the entoglossal process is also protruding from the mouth. During transport of prey, however, the tongue and hyoid are protracted within the oral cavity, but do not leave it. Electromyographic investigation of one of the hyoid retractor muscles (*m. sternohyoideus*)

during the two behaviors shows strong differences in the activation patterns; whereas during transport the hyoid retractors are activated about 300 ms before maximal gape (Herrel et al. 1997a), during prey capture the same muscle is activated immediately before, or at, maximal gape (Herrel et al. 1995). Although this difference seems puzzling at first sight, investigations into the physiological properties of the hyoid retractors may shed light on the observed differences in activation pattern.

Physiological experiments show that at resting length the muscle can only generate about 50% of its maximal force, a situation similar to that observed for the tongue retractor muscle in these lizards (Herrel et al. 2002). As the muscle is stretched, however, it is pulled onto the plateau of its length-tension curve that lies at about 120–150% of resting length. As the hyoid retractors originate on the pectoral girdle, the muscle is stretched to different degrees during both prey capture and prey transport. Indeed, whereas during prey capture the hyoid retractors are stretched to about 150% of their resting length, during prey transport the hyoid retractors are stretched to about 120–130% of resting length. Given the physiological properties of the muscle, this implies that the muscle can optimally contract upon prey contact during prey capture and work largely on the plateau of its length-tension curve as it is pulling back the hyoid and tongue with adhering prey. During transport, however, the tongue is stretched less and only just reaches the plateau of its length-tension curve. The long preactivation of the muscle as the hyoid is being pulled forward (i.e., lengthening activation) means that the muscle is undergoing an eccentric contraction. This may help it to contract forcefully despite operating on the ascending limb of the length-tension diagram, and may allow the muscle to behave with spring-like properties (Lindstedt et al. 2001, 2002). These data illustrate how highly divergent activation patterns may be used to execute the same functional task: forceful retraction of hyoid and tongue with adhering prey.

One other example illustrating the relevance of understanding the mechanical context of the activity of a muscle comes from a recent study of the role of the distal forelimb muscles during walking on narrow substrates in arboreal frogs of the genus *Phyllomedusa* (Manzano et al. in press). In species of this genus, the forearm muscles are highly individualized and appear to control each digit individually. High speed video and cineradiographic recordings show how the frogs wrap their hands around narrow branches while walking upon them. This allows them to generate a stabilizing moment at the hands and feet and prevents them from toppling sideways as the branches are much narrower than the width of the body. Electromyographic recordings of one of the major hand and finger flexors, the *m. flexor digitorum communis longus* shows that this muscle is active during the stance phase in the two species of arboreal frog studied. This muscle may thus actively contribute to the establishment of the gripping posture of the hand (Fig. 5). Moreover, experiments show that upon stimulation of the muscle a pronounced flexion at the wrist and digits is observed in both *Litoria* and *Phyllomedusa*. However, full closure of the hand is not observed.

In *Phyllomedusa*, a superficial hand muscle, the *m. palmaris profundus*, actually inserts onto the tendon of the *m. flexor digitorum communis longus*, and upon stimulation, pulls the tendon of the latter muscle laterally for about 2–3 mm. Stimulation of this muscle by itself does not, however, cause any flexion at the wrist or digits. Consequently, it can be considered as not contributing to flexion of the wrist and finger. Yet, electromyographic recordings show that this muscle is active *in vivo* during the stance phase in *Phyllomedusa*. Insights into its functional role come from an experiment in which a combined stimulation of both the *m. palmaris profundus* and the *m. flexor digitorum communis longus* was performed. Because the *m. palmaris profundus* pulls on the tendon of the *flexor digitorum communis longus* it actually increases the moment arm and kinematic advantage of the latter that results in full closure of the hand upon stimulation. Consequently, the electromyographic data show how the frog actively changes the moment arm of the *m. flexor digitorum communis longus* by activating the *m. palmaris profundus* to generate a grip. Thus, although no changes in the activity pattern of the muscle occurred in the evolution towards a specialized grip in *Phyllomedusa*, this has gone hand in hand with the cooption of an additional muscle. Without understanding the context of the activity patterns of the muscle, it would be impossible to understand the musculoskeletal control of the system and to appreciate the evolution of the motor control of the system.

Insights into motor control

Electromyographic data show that motor control is dynamic and complex. Activity patterns of muscles can vary within a ''single'' muscle, defined anatomically as, e.g., masseter or temporalis. Muscle activity patterns can vary with the functional demands imposed upon the muscle and the mechanical context within which it is operating. In the context of this flexibility, the fact that temporal components of the activation patterns of muscle sometimes appear to be conserved across major changes in morphology and mechanics is of interest

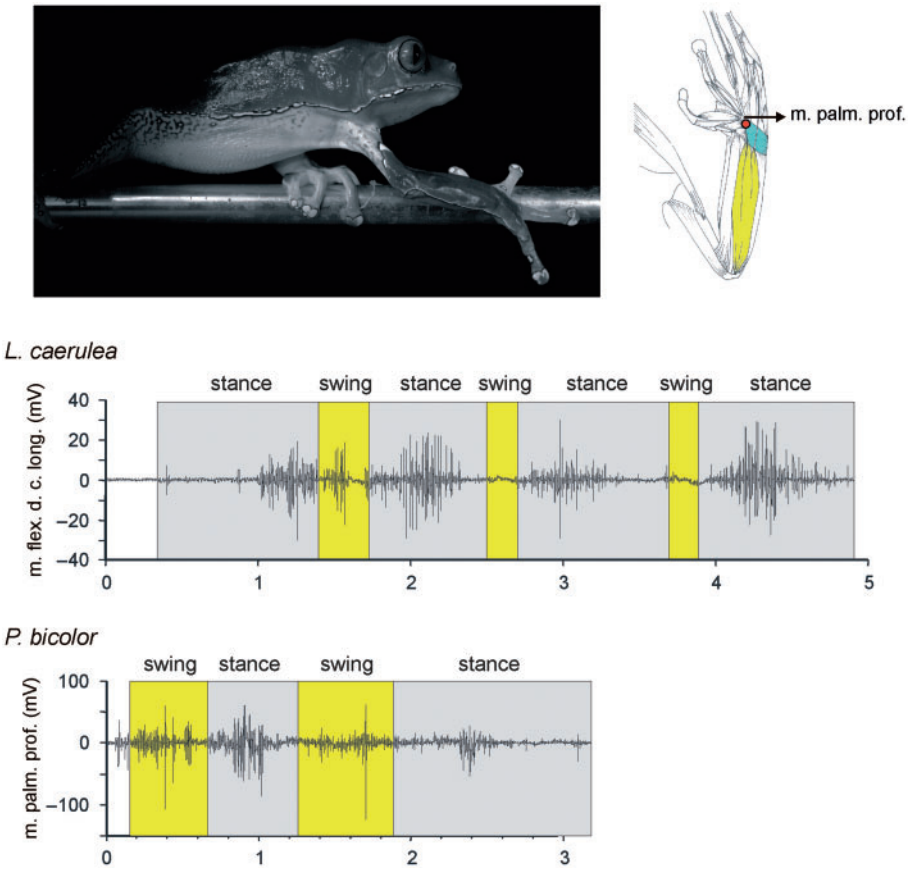

Fig. 5 Top left: image taken from a high-speed movie of a *P. bicolor* walking on a narrow branch. Note how the fingers are extended (right hand) and subsequently wrapped around the branch in a grasping motion. Top right: schematic drawing illustrating the flexor muscles of the hand and fingers in *P. bicolor*. The *m. flexor digitorum communis longis* is in yellow and the *m. palmaris profundus* is in blue. Activity of the *m. palmaris profundus* pulls the tendon of the *m. flexor digitorum communis longus* 2–3 mm to the right, thus changing the moment, arm and the action of the muscle around *the wrist joint* (red circle). Bottom: representative original traces of the *m. flexor digitorum communis longus* in *L. caerulea*, and the *m. palmaris profundus* during locomotion in *P. bicolor*. The *m. flexor digitorum communis longus* is active during stance in all arboreal frogs, including *P. bicolor*. In this species, however, the *m. palmaris profundus* is active during stance as well and thus affects the function of the *m. flexor digitorum communis longus in vivo* and allows *P. bicolor* to close its hand more fully than can any other frog studied to date.

(Jenkins and Goslow 1983; Goslow et al. 1989; Dial et al. 1991; Wainwright 2002; Larson and Stern 1989). However, the conservation of muscle activation patterns and their apparent constraint in some cases may be largely due to the intrinsic capacity of the nervous system to modulate activation patterns, depending on the functional task. The presence of asymmetrical activation in systems with mechanical symmetry (this article; Gerry et al. this volume) and the evolution of derived activation patterns (Grubich 2000; Alfaro et al. 2001), which may even evolve convergently in systems with similar mechanical demands (Konow and Sanford 2008), suggests no intrinsic constraint on the evolution of motor patterns. Moreover, distinguishing between stabilizing selection on a successful control circuit or constraint on the evolution of the control systems is intrinsically difficult (see also Smith 1994). Our data clearly demonstrate that care has to be taken in comparing motor patterns by focusing on functionally similar tasks, and that an understanding of the mechanics of the muscle is crucial for interpreting the control of the musculoskeletal system studied. Given these limitations, EMG appears to be a powerful tool for investigating the evolution of motor control in complex integrated systems such as the vertebrate feeding and locomotory systems.

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