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# Serotonin modulates muscle function in the medicinal leech *Hirudo verbana*

**Shannon P. Gerry* and David J. Ellerby**

*Department of Biological Sciences, Wellesley College, 106 Central Street, Wellesley, MA 02481, USA*
**Author for correspondence (sgerry@wellesley.edu).*

**The body wall muscles of sanguivorous leeches power mechanically diverse behaviours: suction feeding, crawling and swimming. These require longitudinal muscle to exert force over an extremely large length range, from 145 to 46 per cent of the mean segmental swimming length. Previous data, however, suggest that leech body wall muscle has limited capacity for force production when elongated. Serotonin (5-HT) alters the passive properties of the body wall and stimulates feeding. We hypothesized that 5-HT may also have a role in allowing force production in elongated muscle by changing the shape of the length–tension relationship (LTR). LTRs were measured from longitudinal muscle strips *in vitro* in physiological saline with and without the presence of 10 μM 5-HT. The LTR was much broader than previously measured for leech muscle. Rather than shifting the LTR, 5-HT reduced passive muscle tonus and increased active stress at all lengths. In addition to modulating leech behaviour and passive mechanical properties, 5-HT probably enhances muscle force and work production during locomotion and feeding.**

**Keywords:** serotonin; length–tension; obliquely striated muscle

## 1. INTRODUCTION

Sanguivorous leech body wall muscles have an unusually diverse functional repertoire, powering crawling, swimming and suction feeding behaviours [1]. These have distinct kinematic and motor control patterns: dorsoventral undulation of the body axis during swimming; cycles of body elongation, anterior anchoring, then posterior retraction during crawling; and antero-posterior waves of contraction to create suction pressure during feeding [2]. Feeding contractions are maintained, and locomotor movements rapidly resumed [3] despite the extreme body volume changes that stretch the body wall during feeding. This kinematic variability and maintenance of function during body distension suggests that leech body wall muscle is capable of exerting force and performing mechanical work across an unusually wide range of muscle lengths.

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Leech muscle is obliquely striated: adjacent sarcomeres are displaced along the long axis of the muscle, creating a diagonal sarcomere pattern in longitudinal sections [4]. This muscle is present in a number of invertebrate groups, and may have a broader length–tension relationship (LTR) than that found in vertebrate cross-striated muscle [4]. The relationship for leech muscle is asymmetrical, however, allowing maintenance of force production at shorter relative lengths than in cross-striated muscle, yet having similarly impaired force production when elongated [4]. Consequently, how can leech body wall muscle remain functional during, and immediately after feeding?

Hormonal modulation of body wall mechanical properties may provide an explanation. Serotonin (5-HT) is important in the initiation and control of feeding behaviour in sanguivorous leeches [2] and may increase the compliance of body wall tissue [1,5,6] to accommodate body distension. No data are available, however, concerning the effects of 5-HT on the LTR, or that can place the LTR in context with the *in vivo* strain of the body wall muscle during locomotion and feeding.

We hypothesized that the elevated 5-HT levels associated with feeding were essential for preserving effective mechanical function of the longitudinal body wall muscles during the distension created by a large blood meal, by shifting the muscle LTR to support force production at increased muscle strains. We tested this using sonomicrometry to quantify the longitudinal strain of body segments during swimming, crawling and feeding in the medicinal leech. These *in vivo* operating strains have been compared with the LTR of leech longitudinal body wall muscle determined *in vitro* both with and without elevated 5-HT levels.

## 2. MATERIAL AND METHODS

### (a) *Experimental animals*

Medicinal leeches (*Hirudo verbana*) were purchased from a commercial supplier (Leeches USA Ltd., Westbury, NY, USA) and maintained on a 12 L : 12 D cycle at 21°C in deionized water with 0.75 g $l^{-1}$ of aquarium salt (Aquarium Salt, Aquarium Pharmaceuticals, Chalfont, PA, USA) added.

### (b) In vivo *experiments*

Longitudinal body segmental strain of five fasted leeches was measured using 0.7 mm sonomicrometry transducers (Sonometrics Corporation, London, Ontario, Canada) secured dorsally at the anterior and posterior margins of a body segment by cyanoacrylate. Sonomicrometry uses the transit time of an ultrasonic pulse to detect the relative spacing of transducer pairs. Segmental length change data were collected using a Sonometrics TRX Series Sonomicrometer (Sonometrics Corporation). Segmental strains were calculated relative to average swimming strain, $L_s$.

Segmental strains were recorded during swimming and crawling in a 20 l tank of 21°C water, and while the leeches fed from an apparatus described in Claflin *et al.* [3]. Briefly, a tissue bath containing defibrinated sheep blood (Product no.DSB500, Hemostat Laboratories, Dixon, CA, USA) at 38°C was introduced to the experimental tank. The leeches attached via their anterior sucker to an access port covered with Parafilm and fed until sated.

### (c) In vitro *experiments*

Six leeches were anesthetized by placing them on ice. The dorsal portion of three adjoining body segments was excised and placed in saline containing (in millimolar): NaCl, 115.0; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 2.0; and HEPES, 10.0; pH 7.4 at 21°C, then divided into narrow longitudinal strips, of which four per leech were typically used for mechanical measurements. The ends of these were placed in aluminium foil clamps leaving only the middle segment exposed, and

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Table 1. Strain amplitudes and strain rates during crawling and feeding. Data are shown as mean ± s.d., $n = 5$. Superscript letters denote homogeneous subsets established by ANOVAs and Tukey post hoc tests ($p < 0.05$). Early and late feed strain data were collected during the first and last 2 min of feeding, respectively. Mid feed strains were collected at the midpoint of feeding.

| behaviour | maximum length (% $L_s$) | minimum length (% $L_s$) | shortening rate ($L_s$ $s^{-1}$) |
|---|---|---|---|
| crawling | $107.8 \pm 3.9^{a}$ | $46.0 \pm 5.9^{a}$ | $0.72 \pm 0.31^{a}$ |
| early feed | $99.8 \pm 9.3^{b}$ | $57.2 \pm 10.5^{b}$ | $0.65 \pm 0.13^{a,b}$ |
| mid feed | $145.2 \pm 16.1^{c}$ | $96.6 \pm 23.4^{c}$ | $0.50 \pm 0.14^{b}$ |
| late feed | $142.5 \pm 25.5^{c}$ | $101.4 \pm 25.6^{c}$ | $0.50 \pm 0.34^{b}$ |

secured with cyanoacrylate. One-half of the preparations were placed in saline containing 10 µM 5-HT (Sigma) solution.

LTRs for the non-5-HT and 5-HT preparations were quantified using a muscle ergometer (300B-LR, Aurora Scientific, Aurora, Ontario, Canada). Passive tonus and peak force in response to a 1 ms pulse, 80 Hz, 200 ms total duration electrical stimulus delivered via platinum electrodes parallel to the muscle strips were recorded in 20 per cent $L_s$ increments from 40 to 160 per cent $L_s$ with a 3 min relaxation time at each increment. Stimulus current was adjusted for each muscle strip to elicit maximal active force production. The force and position data were captured to a PC via a 604A A to D interface (Aurora Scientific) and a PCI A to D card (PCI-6503, National Instruments, Austin, TX, USA).

### (d) *Statistical analysis*

A two-way ANOVA (SPSS, v. 17.0; SPSS Inc., Chicago, IL, USA) was used to test for differences in peak strain, minimum strain and shortening rate between crawling and feeding. A three-way ANOVA with leech, muscle length and treatment as fixed factors tested for the effects of serotonin on the passive and active properties of the muscle LTR.

## 3. RESULTS

Swimming produced cyclical strain changes (figure 1) with a peak-to-peak amplitude of $16.8 \pm 4.2\%$ $L_s$ at a frequency of $2.58 \pm 0.32$ Hz (mean ± 1s.d.). Crawling and feeding strain patterns were qualitatively similar but shifted to a significantly higher strain range during feeding (figure 1, table 1 and the electronic supplementary material, table S1).

Exposure to 5-HT was associated with increased stress during stimulation ($p < 0.001$; figure 2*a*; the electronic supplementary material, table S2). Individual leeches differed in the magnitude of their response to 5-HT ($p = 0.007$), yet all individuals followed the same trend. Residual tonus increased with muscle length and was significantly lower overall in the muscle strips exposed to 5-HT ($p = 0.001$; figure 2*b* and the electronic supplementary material, table S3). Exposure to 5-HT did not alter the shape of the LTRs for active or passive stress ($p > 0.05$), but shifted them relative to the *y*-axis.

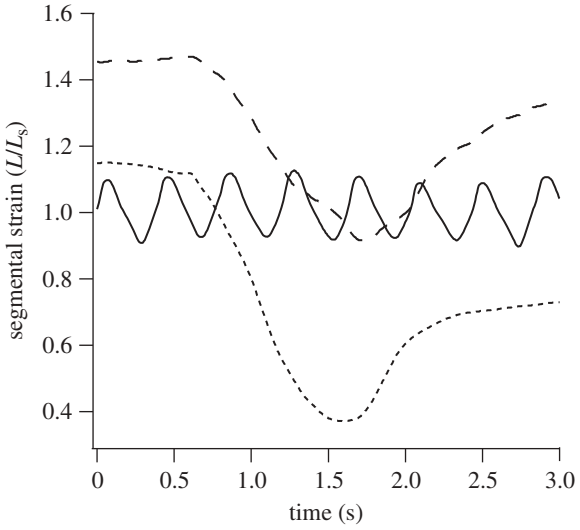


Figure 1. Representative longitudinal muscle strains during swimming (solid line), crawling (dotted line) and suction feeding (dashed line) behaviours. The traces correspond to the shortening phases of crawling and feeding because these are directly relevant to the LTR. Strain is expressed as a percentage of mean swimming length ($L_s$).

## 4. DISCUSSION

Leech longitudinal muscles exert force across a remarkably wide length range in comparison to the muscle of other organisms (figure 1 and table 1). The *in vivo* operating range is approximately 75–240% of $L_0$, the length at which maximal force was exerted (figure 2). Exposure to 5-HT decreases residual tonus and increases force production in response to stimulation (figure 2). Contrary to our initial hypothesis, force production increased at all muscle lengths, not only at the higher segmental strains associated with feeding. This would probably enhance muscle work output during locomotion and feeding.

The LTR was much broader than previously measured in leech muscle (figure 2). This may reflect methodological differences, as previous data were obtained from the whole dorsal body wall of a leech [4], rather than a narrow longitudinal strip from a single body segment. The presence of intact circular and longitudinal muscles and the difficulty of fully and evenly stimulating a large piece of muscle tissue may account for the performance differences. Muscle strip LTRs from earthworm longitudinal muscle are similar to those obtained in this study [7]. This may be the typical pattern for annelid longitudinal muscle measured at the single segment level.

Our data fit the predictions for broad obliquely striated muscle LTRs derived from images of changing sarcomere arrangement in relation to muscle length [4,8]. The absence of Z-discs potentially allows greater elongation than in cross-striated muscle through the shearing of adjacent myosin filaments [9–11]. In addition, cross-bridge formation may be maintained during extreme elongation by actin filaments 'changing partners' from a myosin filament with which they no longer overlap to other adjacent myosin filaments

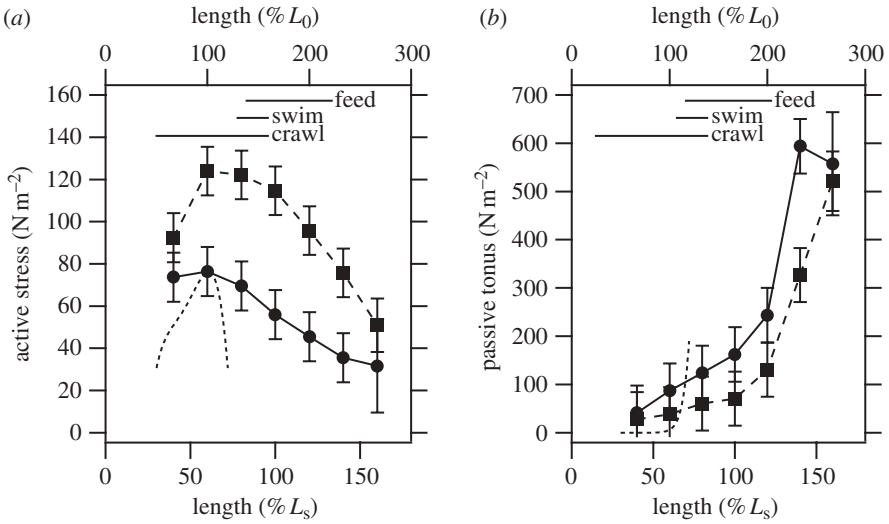


Figure 2. Modulation of the longitudinal muscle length–tension relationship by serotonin (5-HT) when (*a*) stimulated or (*b*) during passive tonus. Solid and dashed lines indicate data obtained in the absence and presence of 10 μM 5-HT, respectively. Data shown are means ± s.e.m. Horizontal bars show *in vivo* length range. Dotted lines show data from Miller [4] plotted in relation to $L_0$ and scaled so that peak active stresses in the absence of 5-HT coincide.

with which cross-bridge formation is possible [4,8]. The mechanisms that reduce residual tonus and increase active force production in the presence of 5-HT are less clear in comparison.

The persistent tonus may be generated by a 'catch' mechanism similar to that described in *Mytilus* anterior byssus retractor muscle (ABRM). In the ABRM, an accessory protein—twitchin—acts as a tether between the thick and thin filaments, maintaining force production at low metabolic cost with minimal cross-bridge cycling [12]. Twitchin phosphorylation in the presence of 5-HT relaxes the catch state [13–15]. Twitchin-like proteins have been isolated from the obliquely striated muscle of other invertebrates, including *Hirudo* [16]. Also, several invertebrates show reduced tonus in the presence of 5-HT [7,17,18]. Together, this provides circumstantial evidence that a 5-HT controlled catch mechanism may be operating in *Hirudo*, although confirmation would require detailed investigation of the differential response of active contraction and tonus to treatments that eliminate cross-bridge formation, and the binding properties of *Hirudo* 'twitchin' in relation to its phosphorylation state.

No comparable data are available for *Hirudo* obliquely striated muscle stimulated to elicit maximal force production in the presence of 5-HT. However, 5-HT does enhance force production in the body wall muscle of other annelids during spontaneous [19,20], acetylcholine-induced [17] and electrically stimulated contractions [21]. There is a similar potentiating effect of 5-HT on *Aplysia* muscle [22], associated with a 5-HT-mediated increase in inward calcium currents across muscle cell membranes [23,24]. Confirmation of a similar 5-HT-mediated mechanism in *Hirudo* muscle requires further investigation.

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1 Wilson, R. J. A., Skierczynski, B. A., Meyer, J. K., Skalak, R. & Kristan Jr, W. B. 1996 Mapping motor neuron activity to overt behavior in the leech I. Passive biomechanical properties of the body wall. *J. Comp. Physiol. A* **178**, 637–654.

2 Lent, C. M. & Dickinson, M. H. 1984 Serotonin integrates the feeding behavior of the medicinal leech. *J. Comp. Physiol. A* **154**, 457–471.

3 Claflin, S. B., Pien, C. L., Rangel, E. N., Utz, K. E., Walther, H. V., Wright, A. N. & Ellerby, D. J. 2009 Effects of feeding on medicinal leech swimming performance. *J. Zool.* **277**, 241–247. (doi:10.1111/j.1469-7998.2008.00534.x)

4 Miller, J. B. 1975 The length–tension relationship of the dorsal longitudinal muscle of a leech. *J. Exp. Biol.* **62**, 43–53.

5 Mason, A. & Kristan Jr, W. B. 1982 Neuronal excitation, inhibition and modulation of leech longitudinal muscle. *J. Comp. Physiol.* **146**, 527–536. (doi:10.1007/BF00609449)

6 Tian, J., Iwasaki, T. & Friesen, W. O. 2007 Muscle function in animal movement: passive mechanical properties of leech muscle. *J. Comp. Physiol. A* **193**, 1205–1219. (doi:10.1007/s00359-007-0278-y)

7 Tashiro, N. & Yamamoto, T. 1971 The phasic and tonic contraction in the longitudinal muscle of the earthworm. *J. Exp. Biol.* **55**, 111–122.

8 Lanzvecchia, G., De Eguileor, M. & Valvassori, R. 1985 Superelongation in helical muscles of leeches. *J. Muscle Res. Cell Motil.* **6**, 569–584. (doi:10.1007/BF00711915)

9 Rosenbluth, J. 1967 Obliquely striated muscle. III. Contraction mechanism of Ascaris body muscle. *J. Cell Biol.* **34**, 15–33. (doi:10.1083/jcb.34.1.15)

10 Mill, P. J. & Knapp, M. F. 1970 The fine structure of obliquely striated body wall muscles in the earthworm, *Lumbricus terrestris* Linn. *J. Cell Sci.* **7**, 233–261.

11 D'Haese, J. & Ditgens, A. 1987 Studies on isolated obliquely striated muscle cells- shearing mechanism implicated in contraction without Z-rods. *Eur. J. Cell Biol.* **44**, 79–85.

12 Hooper, S. L., Hobbs, K. H. & Thuma, J. B. 2008 Invertebrate muscles: thin and thick filament structure; molecular basis of contraction and its regulation, catch and asynchronous muscle. *Prog. Neurobiol.* **86**, 72–127. (doi:10.1016/j.pneurobio.2008.06.004)

13 Funabara, D., Kanoh, S., Siegman, M. J., Butler, T. M., Hartshorne, D. J. & Watabe, S. 2005 Twitchin as a regulator of catch contraction in molluscan smooth muscle. *J. Muscle Res. Cell Motil.* **26**, 455–460. (doi:10.1007/s10974-005-9029-2)
14 Butler, T. M., Mooers, S. U. & Siegman, M. J. 2006 Catch force links and the low to high force transition of myosin. *Biophys. J.* **90**, 3193–3202. (doi:10.1529/biophysj.105.077453)
15 Galler, S., Litzlbauer, J., Kross, M. & Grassberger, H. 2010 The highly efficient holding function of the mollusc 'catch' muscle is not based on decelerated myosin head cross-bridge cycles. *Proc. R. Soc. B* **277**, 803–808. (doi:10.1098/rspb.2009.1618)
16 Nave, R. & Weber, K. 1990 A myofibrillar protein of insect muscle related to vertebrate titin connects Z band and A band: purification and molecular characterization of invertebrate mini-titin. *J. Cell Sci.* **95**, 535–544.
17 Alvarez, M. C., Del Castillo, J. & Sanchez, V. 1969 Pharmacological responses of the dorsal longitudinal muscle of *Sabellastarte magnifica*. *Comp. Biochem. Physiol.* **29**, 931–942. (doi:10.1016/0010-406X(69)90995-5)
18 Reinitz, C. A. & Stretton, A. O. W. 1996 Behavioral and cellular effects of serotonin on locomotion and male mating posture in *Ascaris suum* (Nematoda). *J. Comp. Physiol. A* **178**, 655–667.
19 Gardner, C. R. & Cashin, C. H. 1975 Some aspects of monoamine function in the earthworm (*Lumbricus terrestris*). *Neuropharmacology* **14**, 493–500. (doi:10.1016/0028-3908(75)90053-2)
20 Csoknya, M., Takacs, B., Koza, A., Denes, V., Wilhelm, M., Hiripi, L., Kaslin, J. & Elekes, K. 2005 Neurochemical characterization of nervous elements innervating the body wall of earthworms (Lumbricus, Eisenia): immunohistochemical and pharmacological studies. *Cell Tissue Res.* **321**, 479–490. (doi:10.1007/s00441-005-1134-4)
21 Gardner, C. R. 1981 Effects of neurally active amino acids and monoamines on the neuromuscular transmission of *Lumbricus terrestris*. *Comp. Biochem. Physiol.* **68**, 85–90. (doi:10.1016/0306-4492(81)90041-1)
22 McPherson, D. R. & Blankenship, J. E. 1991 Neural control of swimming in *Aplysia brasiliana* III. Serotonergic modulatory neurons. *J. Neurophysiol.* **66**, 1366–1379.
23 Březina, V., Evans, C. G. & Weiss, K. R. 1994 Enhancement of Ca current in the accessory radula closer muscle of *Aplysia californica* by neuromodulators that potentiate its contractions. *J. Neurosci.* **14**, 4394–4411.
24 Laurienti, P. J. & Blankenship, J. E. 1997 Serotenergic modulation of a voltage-gated calcium current in the parapodial swim muscle from *Aplysia brasiliana*. *J. Neurophysiol.* **77**, 1469–1502.