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Serotonin as an integrator of leech behavior and muscle mechanical performance

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ABSTRACT

The obliquely striated muscle in the leech body wall has a broad functional repertoire; it provides power for both locomotion and suction feeding. It also operates over an unusually high strain range, undergoing up to threefold changes in length. Serotonin (5-HT) may support this functional flexibility, integrating behavior and biomechanics. It can act centrally, promoting motor outputs that drive body wall movements, and peripherally, modulating the mechanical properties of body wall muscle. During isometric contractions 5-HT enhances active force production and reduces resting muscle tone. We therefore hypothesized that 5-HT would increase net work output during the cyclical contractions associated with locomotion and feeding. Longitudinal strains measured during swimming, crawling and feeding were applied to body wall muscle *in vitro* with the timing and duration of stimulation selected to maximize net work output. The net work output during all simulated behaviors significantly increased in the presence of 100 μM 5-HT relative to the 5-HT-free control condition. Without 5-HT the muscle strips could not achieve a net positive work output during simulated swimming. The decrease in passive tension associated with 5-HT may also be important in reducing muscle antagonist work during longitudinal muscle lengthening. The behavioral and mechanical effects of 5-HT during locomotion are clearly complementary, promoting particular behaviors and enhancing muscle performance during those behaviors. Although 5-HT can enhance muscle mechanical performance during simulated feeding, low *in vivo* activity in serotonergic neurons during feeding may mean that its mechanical role during this behavior is less important than during locomotion.

1. Introduction

Obliquely striated muscle, characterized by Z-elements arranged at an oblique angle to the long axis of the cell, powers movement in a wide range of invertebrate phyla (e.g., Rosenbluth, 1967; Bone and Ryan, 1974; Ward et al., 1986; Norenburg and Roe, 1997). It can exert force across a much wider range of lengths than vertebrate, cross-striated muscle (Miller, 1975; Milligan et al., 1997; Gerry and Ellerby, 2011), likely through the shearing of adjacent myosin filaments (Rosenbluth, 1967; Lanzavecchia et al., 1985; D'Haese and Ditgens, 1987). Therefore, this muscle type can drive movements that require extremely high muscle strains. For example, the obliquely striated body wall musculature of sanguivorous leeches powers both suction feeding, during which leeches may undergo a tenfold increase in volume (Lent et al., 1988; Claflin et al., 2009), and locomotion which can involve threefold changes in body length (Stern-Tomlinson et al., 1986; Gerry and Ellerby, 2011).

A broad isometric length–tension relationship in itself is unlikely to explain the ability of leech muscle to maintain force and work production throughout the *in vivo* strain range. Body wall muscle strips maintain substantial passive tension (Tian et al., 2007; Gerry and Ellerby, 2011), and when stimulated a persistent tonus can elevate stress levels for over 30 s post-stimulation (Miller and Aidley, 1973; Gerry and Ellerby, 2011). Persistent residual forces mean that substantial work would have to be done by antagonistic muscle layers or muscle in adjacent body segments to elongate muscle fibers that drive cyclical movements. This would limit performance by reducing the amount of net work available to transfer momentum to the surrounding water during swimming, change the shape of body segments during crawling and create pressure gradients to move blood during suction feeding.

The mechanical performance of body wall muscle may be enhanced by serotonin (5-HT). 5-HT influences leech behavior both through modulation of motor pattern generation by the central nervous system and peripheral effects on tissue function (Mason

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and Kristan, 1982; Nusbaum and Kristan, 1986; Lent et al., 1988; Tian et al., 2007; Brodfuehrer et al., 2008; Gaudry et al., 2010). Elevated 5-HT levels are associated with pre-feeding search behavior, increased swimming activity and increased biting frequency (Willard, 1981; Lent and Dickinson, 1984; Lent et al., 1988; Wilson et al., 1996a). 5-HT also enhances active force production by the body wall muscle while reducing the persistent tonus (Wilson et al., 1996a; Tian et al., 2007; Gerry and Ellerby, 2011). The central and peripheral effects of 5-HT may therefore be complementary (Mason and Kristan, 1982): promoting certain behaviors by modulating motor outputs from the central nervous system while also enhancing muscle work output during those behaviors.

We hypothesized that the elevated 5-HT levels associated with feeding behavior would enhance the work output of body wall muscle during locomotion and feeding by both increasing the work done during muscle shortening, and reducing negative work, the amount of work required to stretch the muscle during cyclical contractions. In order to test this, strips of leech longitudinal muscle were subjected to strain cycles that mimicked those experienced *in vivo* during locomotion and feeding, and stimulated to maximize work output during those cycles. Performance was characterized both in the presence of 5-HT at levels approximating those *in vivo* during the pre-feeding search phase and feeding, and using a 5-HT-free control.

Data are available concerning the effects of stimulus timing on force production by leech body wall strips during simulated swimming (Chen et al., 2011b), but none are available for the mechanical performance of leech muscle across the entire functional repertoire of feeding and locomotor behaviors, or for the influence of 5-HT on mechanical performance under *in vivo* strains. Although the contractile properties of obliquely striated muscle have been characterized in a number of invertebrate species (Tashiro, 1971; Miller, 1975; Thompson et al., 2008), few data are available concerning *in vivo* muscle strains (Rosenbluth et al., 2010; Thompson et al., 2010; Chen et al., 2011b) and the mechanical performance of the muscle when subjected to these strains (Chen et al., 2011b). The present study will provide insight into the dynamic, *in vivo* function of obliquely striated muscle as a power source, and the potential role of 5-HT as an integrator of behavior and biomechanics.

2. Materials and methods

2.1. Experimental animals

Medicinal leeches *Hirudo verbana* (Siddall et al., 2007) were purchased from a commercial supplier (Leeches USA, Westbury, NY, USA) and maintained on a 12:12 light:dark cycle at 21 °C in deionized water with 0.75 g l^{-1} of aquarium salt (Aquarium Pharmaceuticals, Chalfont, PA, USA) added. 7 leeches (mass 1.5–2.0 g) were used for data collection.

2.2. Measurement of segmental lengths during swimming

Sonomicrometry was used to determine the mean length of a mid-body segment during swimming (L_s) for each leech used for muscle mechanical performance measurements. This served as a length standard for subsequent *in vitro* experiments. Sonomicrometry uses the transit time of ultrasound between pairs of piezoelectric transducers to determine their spacing.

Fasted leeches were anesthetized by placing them on ice. Two 0.7 mm diameter transducers (Sonometrics Corp., London, ON, Canada) were attached on the dorsal midline of the leech using cyanoacrylate adhesive (Krazy glue, Columbus, OH, USA), at the anterior and posterior margins of a mid-body segment. The mid-body segments are the primary source of mechanical power during undulatory swimming in leeches (Chen et al., 2011a). The leech was placed in a tank of aquarium salt solution (0.75 g l^{-1}) where normal locomotor behavior was rapidly resumed. Mean segmental lengths were calculated from bouts of steady, straight line swimming with a minimum of 10 swimming cycles, each cycle representing a complete sinusoidal period. On completion of the segmental length measurements leeches were re-anesthetized for removal of the sonomicrometry transducers, assigned unique identifiers and maintained in individual containers.

2.3. Muscle mechanical performance

Leeches were anesthetized and the dorsal portion of three adjoining body segments was excised and placed in saline (pH 7.4 at 21 °C) containing 115.0 mM NaCl, 4.0 mM KCl, 1.8 mM $CaCl_2$, 2.0 mM $MgCl_2$ and 10.0 mM HEPES, then divided into longitudinal strips 0.5 mm in width, of which four per leech were typically used for mechanical measurements. The ends of these were placed in clips made from T-shaped pieces of aluminum foil (Altringham and Johnston, 1994). These were attached to each end of the muscle strips by folding and crimping the arms of the T around the strips, and securing them with cyanoacrylate, leaving only the middle body segment exposed. The remaining free arms of the T served as attachment points to the muscle ergometer. One-half of the preparations were placed in saline containing 100 μM 5-HT solution (Sigma–Aldrich, St. Louis, MO, USA).

The local 5-HT concentration created within the leech longitudinal muscle by the serotonergic Retzius neurons is not known. The serotonergically innervated body wall muscle in another annelid, the earthworm, has a 5-HT concentration of 320 μM (Takács et al., 2007). Exogenous 5-HT applications of 100 μM or more trigger behavioral responses similar to those associated with increased Retzius neuron activity (Willard, 1981; Lent and Dickinson, 1984). The threshold concentration for exogenously applied 5-HT to affect body wall tension is approximately 10 μM (Tian et al., 2007). Preliminary measurements of baseline and peak active stress were made at 5-HT concentrations of 0, 1, 10, 100, 500 and 1000 μM. Peak stress increased and baseline stress declined from 0 to 100 μM. There were no detectable differences in response across the 100–1000 μM range, therefore the minimum concentration eliciting a response was used.

Muscle mechanical properties were quantified using a muscle ergometer (300B-LR; Aurora Scientific, Aurora, ON, Canada), which controls muscle length and stimulus timing while measuring force output. Muscle strips were suspended vertically between the ergometer lever arm and the base of a tissue chamber, attached *via* the aluminum foil clips, and their starting length set to the mean swimming segmental length, L_s. The tissue chamber contained oxygenated leech physiological saline with or without 5-HT at 21 °C. Stimuli were delivered *via* platinum electrodes parallel to the muscle strip. Stimulus current was adjusted to elicit maximal force production. Force and length data were captured to a PC *via* a 604A interface (Aurora Scientific) and a PCI A/D card (PCI-6503; National Instruments, Austin, TX, USA).

Isometric contractile properties were measured using a 1 ms pulse, 80 Hz, 300 ms total stimulus train duration. We recorded the pre-stimulus muscle stress, peak active stress (S_a, peak stress minus the pre-stimulus baseline) and the stress at the start of the persistent, post-stimulation tonus (minus the pre-stimulus baseline) (Fig. 1). Stresses were calculated based on the cross-sectional area of the longitudinal muscle layer after removal of the epidermis and adjacent circular and oblique muscle layers. Longitudinal muscles account for approximately 60% of the total body wall muscle cross-sectional area (Miller and Aidley, 1973). Average stresses in the body wall would therefore be approximately 60% of those reported.

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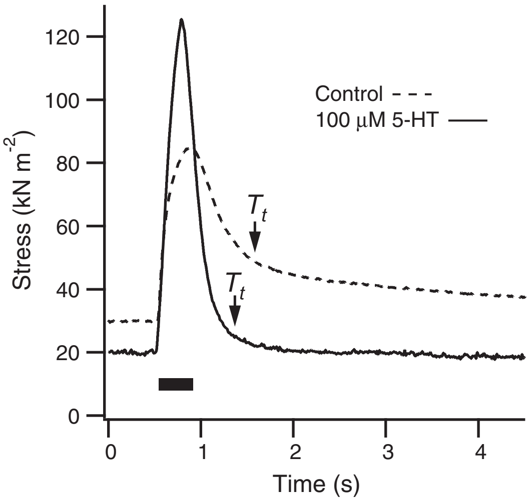

Fig. 1. Representative isometric contractions of leech longitudinal body wall strips in physiological saline. Contractions were in response to a 300 ms stimulus train of 1 ms pulses at 80 Hz. The control contained no 5-HT. The horizontal black bar shows stimulus timing. T_t indicates the onset of the persistent post-stimulation tonus.

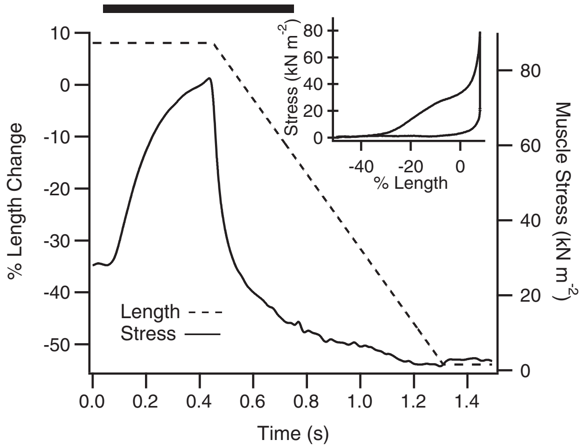

Fig. 2. Representative stress and length traces for leech longitudinal muscle strips during simulated crawling. The horizontal black bar shows stimulus timing. This simulated the retraction phase of crawling where body shortening is driven by activity of the longitudinal muscles. The inset shows the stress–length work loop plot. The area enclosed by the loop represents the net work done during simulated crawling.

In most types of muscle, tissue stress returns to the pre-stimulus baseline relatively rapidly after stimulation ceases. This is not the case for leech body wall muscle, where a significant tonus may persist for more than 30 s post-stimulation (Fig. 1). Similar contractile patterns have been observed in other annelid body wall muscles (Tashiro, 1971; Miller and Aidley, 1973). As a phase of relatively rapid relaxation is followed by slower relaxation during persistent tonus, this point was identified using the rate of change of muscle force. Force traces were differentiated with respect to time, and a relaxation rate of 0.6 S_a s^{-1} taken as the start point of the persistent tonus (Fig. 1). This relaxation rate was approximately equal to that achieved at 90% relaxation in tetani that lacked a persistent tonus in the presence of 5-HT, a relaxation benchmark typically used in muscles with a single relaxation phase.

2.4. Simulated locomotion

Muscle segmental strains during swimming, crawling and feeding have previously been determined (Gerry and Ellerby, 2011). Swimming strain cycles at 21 °C are sinusoidal with a peak-to-peak amplitude of 16.8% L_s and a frequency of 2.58 Hz. Swimming strains were mimicked by applying a sinusoidal strain of this amplitude and frequency to the muscle strips (Josephson, 1985). Segmental strain patterns during crawling and feeding are qualitatively similar. The shortening phase, driven by the longitudinal muscle, has an approximately constant strain rate. The movements in the two behaviors differ in strain range and rate. During crawling, segments shorten from 107.8 to 46.0% of L_s at 0.72 L_s s^{-1}. During feeding, segments shorten from 142.5 to 101.4% of L_s at 0.50 L_s s^{-1} (Gerry and Ellerby, 2011). The shortening phases of crawling and feeding were mimicked by applying a ramp shortening pattern of the appropriate rate and amplitude to the muscle strips (Fig. 2). The precise relationship between segmental strain and activity during locomotion and feeding has not been established *in vivo*. For *in vitro* measurements the timing and duration of stimulation was chosen to maximize the net work output. In a number of cyclically operating muscle systems that power locomotion, the timing of *in vivo* activity approximates that which maximizes work output *in vitro* (e.g., Hammond et al., 1998; Ellerby and Askew, 2007). During ramp shortening, stimulation was started 300 ms before shortening to allow near-maximal force development. During sinusoidal strains, maximal work output was achieved with a stimulus onset 50–80 ms before peak strain and a duration of approximately one-third of the strain cycle. This is similar to the relative duration of motor neuron activity recorded *in vivo* (Kristan and Calabrese, 1976). Stimuli had a 1 ms pulse duration at 80 Hz, as for isometric contractions. During simulated swimming, negative work was calculated as the force–length integral during lengthening. Net work per cycle was calculated as the difference between the shortening force–length integral (total work) and the negative work done during lengthening (Pringle and Tregear, 1969; Josephson, 1985). For ramp shortening cycles, negative work was calculated from the work of shortening during passive cycles, and net work as the difference between total work and negative work.

2.5. Statistical analyses

Data were tested for normality using a Kolmogorov–Smirnov test ($P < 0.05$) and Levene's equality of error variances test ($P < 0.05$). Non-normally distributed data were log-transformed to achieve normality. Means for untransformed data are presented in Tables 1 and 2.

An analysis of variance (ANOVA) was conducted using the general linear model function in SPSS v. 17 (SPSS Inc., Chicago, IL, USA). The presence or absence of 5-HT was included as a fixed factor in the model, and an individual identifier for each leech as a random factor. To account for the use of multiple comparisons, the experiment-wise error rate was adjusted using a sequentially rejective multiple test procedure (Holm, 1979) applying Ryan's *Q*.

Cohen's *d* (Cohen, 1988) was calculated as an indicator of effect size:

$$d = \frac{(m_{\text{5-HT}} - m_c)}{\sigma_{\text{pooled}}},$$

where $m_{\text{5-HT}}$ and m_c were the mean values for the 100 μm 5-HT and control groups, respectively, and σ_{pooled} was the root mean square of their standard deviations.

3. Results

The presence of 5-HT was associated with significant changes in muscle isometric contractile performance in comparison to the 5-HT-free control condition (Fig. 1). Baseline muscle tone before stimulation, force generated during stimulation, and the rate of relaxation after stimulation were all affected. The pre-stimulation baseline stress was higher in the 5-HT-free control. In the presence

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Table 1

Isometric contractile properties of leech longitudinal body wall muscle under 5-HT-free control conditions and with 100 μM 5-HT. Data are shown as means ± 1 SD. $N = 7$. Bold script indicates significance based on Ryan's *Q*. Cohen's *d* is an indicator of effect size based on the difference between means. Cohen (1988) classified effect sizes as small (0.2), medium (0.5) and large (0.8). To allow comparison to data from other muscle tissue, stresses are calculated based on the longitudinal muscle cross-section. This constitutes approximately 60% of total body wall cross-section (Miller and Aidley, 1973).

	Control, no 5-HT	100 μM 5-HT	*F*	*P*	Cohen's *d*
Baseline stress (kN m^{-2})	33.3 ± 20.3	17.6 ± 12.6	12.4	**0.009**	−0.9
Peak active isometric stress (kN m^{-2})	45.1 ± 21.3	97.4 ± 36.6	55.0	**0.001**	1.8
Stress during persistent tonus (kN m^{-2})	21.4 ± 10.9	16.1 ± 18.0	3.9	0.071	−0.4
Persistent tonus: active stress ratio	0.41 ± 0.05	0.17 ± 0.05	51.9	**0.001**	−2.7
Active stress: baseline stress ratio	1.69 ± 0.84	7.35 ± 4.50	19.7	**0.003**	1.8
Time from baseline to peak stress, T_p (s)	0.365 ± 0.039	0.309 ± 0.039	14.3	**0.007**	−1.4
Time from baseline to persistent tonus, T_t (s)	0.838 ± 0.165	0.772 ± 0.104	1.1	0.325	−0.5

Table 2

Work output of leech longitudinal body wall muscle during simulated swimming, crawling and feeding under 5-HT-free control conditions and with 100 μM 5-HT. Data are shown as means ± 1 SD. $N = 7$. Bold script indicates significance based on Ryan's *Q*. Cohen's *d* is an indicator of effect size based on the difference between means. Cohen (1988) classified effect sizes as small (0.2), medium (0.5) and large (0.8). Work values are expressed relative to longitudinal muscle mass after removal of other tissue layers. Longitudinal muscles account for approximately 60% of the tissue cross-section and, assuming equal tissue densities, body wall mass (Miller and Aidley, 1973).

	Control, no 5-HT	100 μM 5-HT	*F*	*P*	Cohen's *d*
Swimming negative work (J kg^{-1})	−7.05 ± 5.23	−1.64 ± 1.78	6.9	**0.033**	−1.4
Swimming net work (J kg^{-1})	−3.41 ± 1.76	11.17 ± 7.09	18.9	**0.005**	2.8
Crawling negative work (J kg^{-1})	−2.83 ± 1.40	−1.44 ± 0.73	7.0	0.057	−1.3
Crawling net work (J kg^{-1})	4.61 ± 2.90	10.33 ± 5.42	14.9	**0.018**	1.3
Feeding negative work (J kg^{-1})	−5.43 ± 4.26	−2.10 ± 0.71	5.6	0.076	−1.1
Feeding net work (J kg^{-1})	3.71 ± 2.21	11.88 ± 6.26	14.0	**0.020**	1.7

of 5-HT, the peak stress reached during stimulation was higher and reached more rapidly than in the control. Without 5-HT, there was a proportionally higher persistent muscle tone after stimulation than in the presence of 5-HT. Isometric contractile performance is summarized in Table 1.

Significant differences in relation to the presence of 5-HT were also detected in muscle work and power output during simulated swimming, crawling and feeding as measured by the work loop technique (Table 2, Figs. 2 and 3). The net work done by the muscle was significantly greater during all simulated strain cycles in the presence of 5-HT. This is largely because the negative work component, representing the work needed to elongate the muscle during cyclical movements, was lower than in the control condition (Table 2). Reduced negative work is associated with a lower pre-stimulation muscle tone, and, in the case of repeated swimming cycles, an increased rate of relaxation (Fig. 1 and Table 1), which both lower the resistance to muscle elongation in the presence of 5-HT.

This was particularly apparent during simulated swimming, where in the 5-HT-free control condition, the stress during muscle

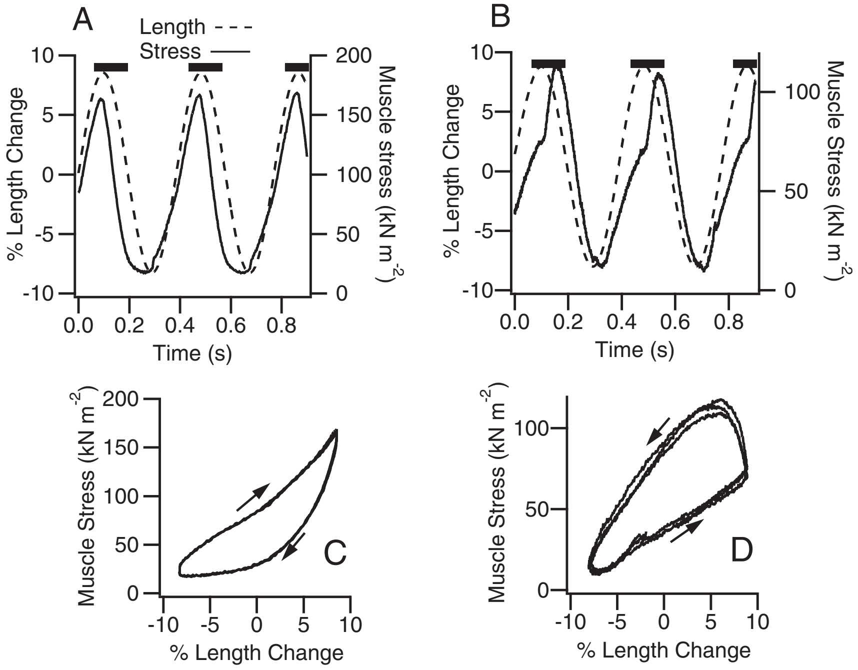

Fig. 3. Representative stress and length traces for leech longitudinal muscle strips during simulated swimming. (A and B) Muscle stress and length change (A) in a 5-HT-free control, (B) with 100 μM 5-HT. The horizontal black bars show stimulus timing. (C and D) Work loop plot of the stress–length relationship (C) for the 5-HT-free control data shown in (A), (D) for the 100 μM 5-HT data shown in (B). Arrows indicate the direction of the work loops. Three loops are superimposed in each plot. Clockwise work loops indicate net negative work by the muscle strip, anti-clockwise loops indicate net positive work.

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lengthening exceeded that during subsequent muscle shortening (Fig. 3A), and as a consequence no net positive work was done by the muscle (Fig. 3C). The reduced resistance to lengthening in the presence of 5-HT (Fig. 3B) supported net positive work output (Fig. 3D). In effect, without 5-HT the muscle was acting as a brake, absorbing work done on the muscle, rather than as a motor doing work to move an external load.

4. Discussion

5-HT has a marked influence on the mechanical performance of leech body wall muscles, enhancing net work and power output during simulated locomotion and feeding. This is achieved through a combination of effects: increased active force production, and reduction of both basal muscle tone and the persistent tonus that can be present after muscle activation (Fig. 1 and Table 1). This increases work output while simultaneously reducing the work required to stretch the muscle during the cyclical length changes typical of locomotion and feeding (Fig. 3 and Table 2).

The behavioral and mechanical effects of 5-HT appear to be complementary, particularly during swimming. Previous predictions, based on the effects of 5-HT on leech body wall isometric properties, that 5-HT may optimize mechanical performance during swimming (Mason and Kristan, 1982) are supported by the present data. 5-HT released from the Retzius neurons acts on the central pattern generator that drives body wall muscle contractions, promoting the swimming motor output (Willard, 1981; Nusbaum and Kristan, 1986). 5-HT simultaneously reduces the work required to elongate longitudinal muscles and maximizes the work done during muscle shortening (Table 2 and Fig. 3). These effects are particularly striking for this mode of movement, as without 5-HT the net work output was negative during simulated swimming; the work required to stretch the muscle during the elongation phase of the cycle exceeded that generated during the shortening phase (Table 2 and Fig. 3C).

Whether this precludes net thrust generation during swimming would depend on the source of power for longitudinal muscle elongation. The dorso-ventral waves of bending associated with swimming are created by waves of longitudinal muscle contraction moving from anterior to posterior along the body axis (reviewed by Brodfuehrer et al., 1995). A phase shift in the timing of the waves between the dorsal and ventral body wall creates bending. The work done to stretch longitudinal muscle fibers is likely done by longitudinal fibers on the opposite side of the body in a similar way to the wave of curvature generated by out-of-phase contra-lateral muscle activity during undulatory swimming in fish (reviewed by Altringham and Ellerby, 1999). The body wall also contains a circular muscle layer, arranged perpendicularly to the longitudinal muscle, and an oblique layer at an intermediate orientation. These can act antagonistically, or partially antagonistically in the case of the oblique layer, to the longitudinal layer, increasing segment length through reduction in segment circumference. Circular muscle activity has been reported during swimming (Baader, 1997) but is less intense than during the extension phase of crawling, likely reflecting partial recruitment of the muscle, and is in phase with that of longitudinal muscle motor neurons (Brodfuehrer et al., 2008). This suggests that antagonistic muscle activity is not a major source of work for longitudinal muscle elongation during swimming.

The role of the circular muscles during swimming may instead be postural, similar to that of the dorso-ventral muscles that flatten the body (Kristan et al., 1974; Ort et al., 1974). Swimming involves a relatively large degree of body elongation. The peak longitudinal strain during a swimming cycle is equal to that reached during the elongation phase of crawling (Gerry and Ellerby, 2011). The circular muscles may therefore elongate the body and contribute to the high internal pressures generated during swimming (Wilson et al., 1996c). Given the limited contribution of mechanical work from muscle antagonists it is unlikely that the longitudinal muscle layer would be able to generate sufficient power to sustain swimming without the influence of 5-HT on mechanical performance.

The mechanical effects described (Tables 1 and 2) would not only enhance the performance of the longitudinal body wall muscles, but reduce the work required from antagonistic muscle groups. This may be particularly important during crawling where circular muscle activity extends the leech forward from an anchor point provided by the posterior sucker. The anterior sucker then grips the substrate, the posterior sucker releases, and the longitudinal muscle system shortens the leech, creating a forward 'step' (see Fig. 1 in Stern-Tomlinson et al., 1986). Reduced passive tension in the longitudinal muscles would require less work to be done by the circular muscle layer during the elongation part of the crawl cycle (Table 2). Retzius cell activity is highest during the elongation phase (Brodfuehrer et al., 2008), suggesting that reduction of passive resistance to antagonist work is an important aspect of 5-HT peripheral function during this behavior.

The functional significance of the 5-HT effects on performance during feeding is less clear. The slow expansion of the body wall is largely achieved incrementally by the continuous pumping of blood by the pharynx into the crop (Lent et al., 1988). Body wall movements during feeding are variable, including waves of peristalsis-like contraction moving both anteriorly and posteriorly along the body axis, and dorso-ventral flexing (Wilson and Kleinhaus, 2000). These may contribute to suction, and also serve to distribute the blood throughout the crop (Lent et al., 1988). Increased longitudinal muscle work output in the presence of 5-HT would potentially enhance these functions, and the reduced passive tension in the body wall would also reduce the work required during pharyngeal pumping to distend the body (Table 2). Retzius cell activity is high during the appetitive phase before feeding, consistent with the behavioral and mechanical role of 5-HT in driving effective locomotion, but falls during feeding (Wilson et al., 1996b). As such, there may be insufficient ongoing 5-HT release into the body wall musculature to alter mechanical performance (Wilson et al., 1996b). A single stimulus of a Retzius cell can, however, reduce the passive tension in the body wall section it innervates for up to 15 min (Mason and Kristan, 1982). High activity levels just before feeding may have persistent effects through at least part of feeding, which typically lasts from 20 to 30 min (Lent et al., 1988; Claflin et al., 2009). Immediately after feeding, Retzius cell activity increases to pre-feeding levels (Wilson et al., 1996a). The distension that accompanies feeding inhibits the swimming motor pattern (Gaudry et al., 2010). Increased urine production during and after feeding rapidly expels blood plasma and reduces body volume (Zerbst-Boroffka et al., 1997; Claflin et al., 2009). High post-feeding 5-HT levels during this process would promote the rapid recovery of locomotor function.

5. Conclusions

Serotonin (5-HT) acts centrally to modulate leech behavior and peripherally to alter the mechanical properties of the muscles that drive body movements. The behavioral and mechanical effects of 5-HT during locomotion are complementary, favoring the motor pattern that drives swimming while supporting the mechanical power output necessary for effective swimming, and reducing the resistance to antagonist work during the extension phase of crawling. Although 5-HT can enhance muscle mechanical performance during simulated feeding, low Retzius cell activity during this behavior may mean that 5-HT's mechanical role during this behavior is less important than during locomotion.

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