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MAPPING MOTOR NEURONE ACTIVITY TO OVERT BEHAVIOUR IN THE LEECH: INTERNAL PRESSURES PRODUCED DURING LOCOMOTION

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Summary

Several behaviour patterns have been studied in the leech at both the kinematic and neuronal levels. However, very little is known about how patterns of motor neurone activity map to actual movements. Internal pressure is an essential biomechanical property in this process, being responsible for producing the rigidity and posture that allow the directed delivery of forces produced by muscle contraction. To obtain a better understanding of the biomechanical processes involved in movement of the leech, we have measured the internal pressure of the animal by placing catheters through the body wall and into the gut of intact animals showing normal patterns of behaviour.

Each type of behaviour had a characteristic pressure waveform. The elongation phase of crawling produced a rapid increase in pressure that peaked when midbody segments were maximally elongated. The pressure produced during the contraction phase of crawling depended on the type of crawl, only inchworm crawling producing a second peak. Whole-body shortening in response to a head poke also produced a pressure peak, but it had a faster rise time. Swimming produced the largest pressure, which was marked by a large sustained increase that fluctuated phasically with undulations of the body. Dual pressure recordings using two catheters demonstrated that pressure was not uniform along the length of the leech, indicating that the body cavity is functionally compartmentalised.

Injecting fluid into the gut *via* a recording catheter allowed us to determine the effects of increasing internal volume on pressure. In line with previous predictions made using an abstract biomechanical model of the leech hydroskeleton, we found that an increase in the volume caused a reduction in the pressure. We are in the process of constructing a more realistic biomechanical model of the leech, based on actual data reported elsewhere. The results in this paper will provide key tests for refining these models.

Key words: internal pressure, biomechanics, muscular hydrostat, locomotion, circular muscles, posture, leech, *Hirudo medicinalis*.

Introduction

Rigidity is required to provide the best posture for the efficient transfer of force from muscle to motion. In organs that contain skeletal tissue, the degree of freedom to move is limited to rotation about joints whose rigidity is controlled by co-activation of antagonistic muscles. In muscular organs that lack hard skeletons, such as the human tongue, the tentacles of an octopus, the trunk of an elephant, the foot of a snail or the body of a leech, the biomechanics is considerably more complex (e.g. Kier and Smith, 1985; Smith and Kier, 1989; Chiel *et al.* 1992). In these structures, internal pressure generated by muscle contraction is solely responsible for rigidity (e.g. Chapman, 1950), reducing the constraints on movement but increasing radically the number of muscles that interact biomechanically. Even the simplest movements are likely to require a carefully orchestrated motor pattern, involving a large number of muscles. In the leech, for example, the local withdrawal reflex that can be triggered by poking a small area of the skin involves the activation of many motor neurones both locally and in neighbouring segments (Kristan *et al.* 1995). How the biomechanical constraints of such a system influence the pattern of motor neurone activity required to generate particular types of behaviour is unknown.

We are addressing this question by developing a biomechanical model of a leech based on experimental data (Wilson *et al.* 1995*a*,*b*; B. A. Skierczynski, R. J. A. Wilson, W. B. Kristan and R. Skalak, in preparation). We have chosen the leech for this study because of the simplicity of its body plan and because the motor neurones that innervate the body wall have been identified (Stuart, 1970; Ort *et al.* 1974; Norris and Calabrese, 1990). Furthermore, the pattern of motor neurone activity underlying a number of distinct types of behaviour, including swimming, crawling, whole-body shortening and the local withdrawal reflex, have been characterised in detail (Brodfuehrer and Friesen, 1986; Kristan

et al. 1988; Friesen, 1989; Baader and Kristan, 1992; Wittenberg and Kristan, 1992; Shaw and Kristan, 1995). By supplying our model with realistic motor neurone input, we intend it to complement previous models of muscular hydrostats that were either qualitative or based on abstracted data (Chiel *et al.* 1992; Wadepuhl and Beyn, 1989). To this end, we have measured the internal pressure of leeches as they perform a range of movements.

The body wall of the leech is composed of the skin and three muscle layers (Mann, 1962): the circular muscles are outermost and act to elongate the animal; the longitudinal muscles are innermost and cause shortening; and an intermediate layer of oblique muscles can act either as longitudinal muscles (when the animal is elongated) or as circular muscles (when the animal is shortened). A fourth set of muscles, the dorso-ventral muscles, span the body cavity from the dorsal to the ventral body wall and produce a flattened body shape when contracted. This morphology is shared by several other muscular hydrostats (e.g. Trueman and Brown, 1976). The cross-sectional area of the circular muscle in any one segment is approximately 5% of the segmental longitudinal muscle cross-sectional area. However, since there are 21 body segments and the circular muscles of the different segments are arranged in parallel (and not in series like the longitudinal muscles), the total cross-sectional areas of these two muscle types are approximately equal. The oblique fibres, in contrast, have less than 10% of the total cross-sectional area of either the longitudinal or circular muscles (Miller and Aidley, 1973; M. Wadepuhl, unpublished data). We have previously characterised the passive properties of the leech body wall which is viscoelastic, exhibiting both resistance to stretch and stress relaxation (Wilson *et al.* 1995*b*). The passive length–tension curves of both the circumferential and the longitudinal axes are relatively flat for most of the physiological range but rise steeply as the strain approaches the physiological maximum. Inherent muscle activation (i.e. activation in the absence of motor neurone input) contributes at least half the passive tension and can be modulated by serotonin (Mason and Kristan, 1982). These properties, along with the morphology of the body, the active properties of the muscle and the neuronal motor patterns that drive them, contribute to the internal pressure.

Several previous studies have recorded the pressure inside the body cavity of actively moving animals such as earthworms, snails and sea hares (Seymour, 1971; Trueman and Brown, 1976; Kuenzi and Carew, 1994). These studies emphasize the importance of internal pressure in locomotion. For example, in the mollusc *Donax serra*, pedal extension and burrowing require a degree of rigidity and are associated with increases in internal pressure. Both types of behaviour produce a distinct waveform of differing magnitude (Trueman and Brown, 1985). In this paper, we demonstrate that different types of behaviour in the leech also produce distinct pressure waveforms. These waveforms provide valuable information regarding the biomechanics of the leech and, since biomechanical models can predict pressure (Wadepuhl and Beyn, 1989; B. A. Skierczynski, R. J. A. Wilson, W. B. Kristan and R. Skalak, in preparation), they also provide a good test of the assumptions of such models.

Materials and methods

Preparation of animals

Leeches *Hirudo medicinalis*, weighing 3–3.5 g, were obtained from Leeches USA and maintained at 15 °C in artificial pond water. The animals were anaesthetised in ice-cold 8% ethanol before dissection. Knots were sewn into the body wall along the dorsal midline to mark the centre annulus of segments 4, 6, 8, 10, 12, 14 and 16. The 8% ethanol was replaced by ice-cold normal saline and a slit, the size of one annulus, was made along the midline through the dorsal body wall. A small incision was made in the gut, through which a catheter was pushed. The catheter consisted of a length of PE50 tubing heated to flare one end and give a tip diameter of approximately 1 mm. The shaft was perforated immediately behind the tip to make blocking less likely. Beads were glued 1 and 2 cm from the tip as markers. The bead nearest the tip acted as a catheter entry marker, the other bead (the catheter distance marker) was used to estimate the location of the tip within the gut. Catheters were filled with normal Ringer before being inserted. Typically, a single catheter was inserted through segment 10. When two catheters were used, one was inserted in segment 9, the other in segment 13. Slits in the body wall were sutured, and the catheters attached *via* the catheter entry marker beads. Operated leeches were left for several hours in normal Ringer to recover.

Pressure measurements

The pressure within the gut of leeches was measured by attaching the free end of the fluid-filled catheter to a pressure transducer (Viggo-Spectramed, CA, USA). Pressure transducers were calibrated using a column of water (Fig. 1) and results are therefore expressed in cmH_2O; $1\,cmH_2O = 0.1\,mN\,mm^{-2} = 98.1\,Pa$. Zero pressure was taken to be the point at which the level of water in the column was equal to that in the observation tank. The output of the pressure transducer was amplified and stored both as a trace on a chart recorder and digitally on a video tape, by way of an analog to digital (A/D) converter. Brief TTL pulses which illuminated a diode next to the chart recorder were also stored on the magnetic tape. Leeches were filmed using a video camera equipped with an auxiliary camera, so that images of the leech, the light-emitting diode and the chart recorder appeared in the same frame. Before frames were recorded, a frame counter was incorporated electronically into each frame (Horita TRG-50). Using the flashing diode and the TTL pulse to synchronise the frame counter with the digital record, the temporal resolution was 1/30 s.

Data analysis

Length and phase measurements were made using NIHimage software. Descriptive statistics are given as

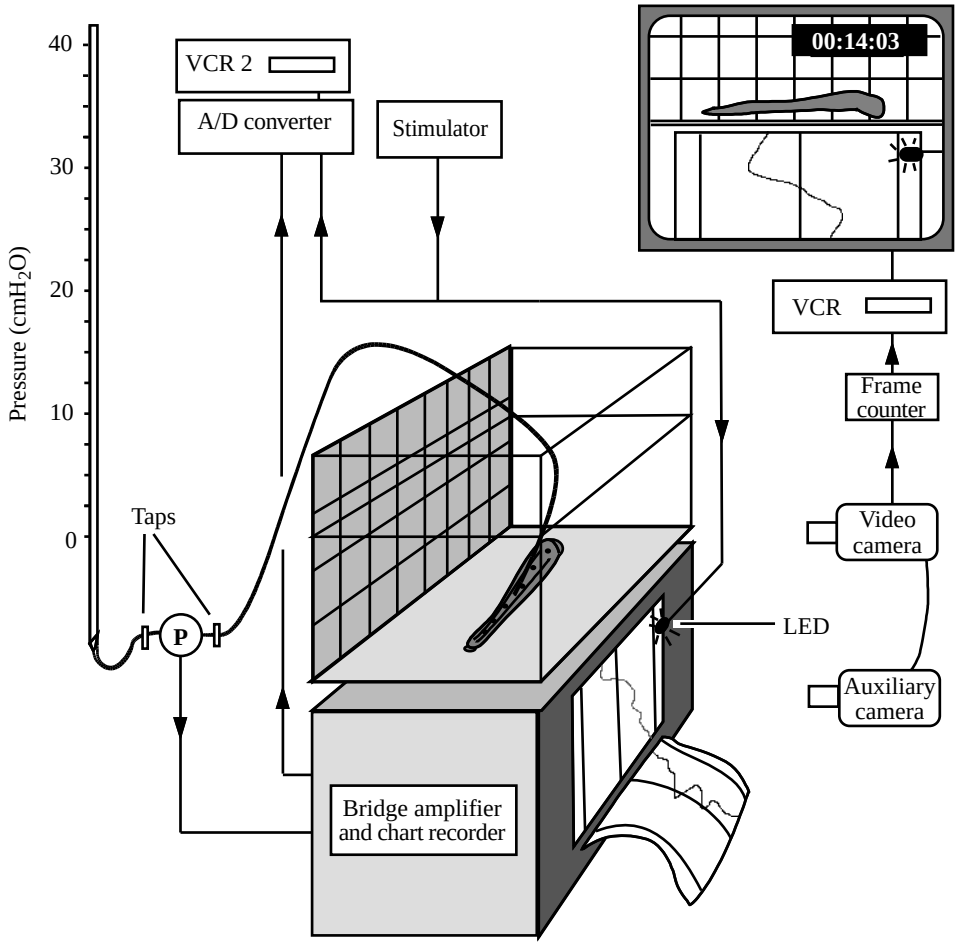


Fig. 1. Method used to record the kinematics and pressure during movement. Typically, for single-site pressure recordings, one end of a length of polyethylene tubing (PE50) was inserted through the dorsal body wall of segment 9 into the gut. The tip of the tube inside the leech usually extended rostrally for about two whole segments. Animals were placed in water in an observation tank and the other end of the tubing was connected to a pressure transducer (P). The pressure transducer was calibrated using a water column. The output of the pressure transducer was amplified and stored both as a trace on a chart recorder and digitally on a video tape by way of an analog to digital (A/D) converter. Brief TTL pulses which illuminated a diode next to the chart recorder were also stored on the magnetic tape. Leeches were filmed using a video camera equipped with an auxiliary camera, so that images of the leeches, the light-emitting diode (LED) and the chart recorder appeared in the same frame. Before frames were recorded, a frame counter was added. Using the flashing diode and the TTL pulse to synchronise the frame counter with the digital record, this arrangement gave a temporal resolution of 1/30 s.

mean ± standard error of the mean throughout. Student's *t*-tests were used to determine significant differences between two groups. When the analysis involved more than two groups, we used analysis of variance (ANOVA) followed by *post hoc* comparison tests to locate differences (the Tukey–Kramer multiple comparison test for comparison across all groups or the Boneferroni multiple comparison test for selected comparisons). Dunn's multiple comparison test was used for nonparametric data. To quantify the effect of injecting volume on pressure, we used multiple regression analysis so that data from all the types of behaviour could be combined.

Results

Several hours after the operation in which a single catheter was implanted into the gut, leeches swam and inchworm-crawled spontaneously and shortened in response to a head poke. In line with behavioural observations on unoperated animals, we observed vermiform crawling only rarely when leeches were under water. The catheter appeared to have little if any effect on the kinematics of these movements. During swimming, for example, animals were able to lift themselves off the bottom of the tank and make progress through the water. More importantly, the undulation of the body retained its characteristic single-wavelength, sine-wave-like shape, indicating that the motor pattern was little perturbed by the tubing inserted into the body.

We performed two sets of control experiments to ensure that fluid did not leak from the cut in the body wall through which the tube was inserted. (Such a leak would lead to an underestimation of the actual pressure.) First, we recorded the pressure in leeches that had fed recently. Despite causing considerable extension of the body of the leech, none of the blood meal leaked from the site of the tube insertion. Second, we injected 1 ml of mineral oil through the catheter into the gut of leeches and were subsequently unable to observe any oil film on the surface of the water. Oil films were occasionally observed after prolonged periods (24 h), but these may be attributed to the leech vomiting.

As a baseline, we quantified the internal pressure immediately preceding movements. Typically, the pressure was of the order of 4.5 cmH_2O. When animals where anaesthetized in 8 % ethanol, this pressure fell to zero, suggesting that the 'resting pressure' was produced by slight muscle activation. Crawling, swimming and shortening produced large increases in pressure, with characteristic waveforms and magnitudes.

Single-site pressure recordings during crawling

Crawling consists of four phases: elongation, in which only the posterior sucker is attached; the transfer of mass, in which both suckers are attached and the centre of mass shifts rostrally (this phase includes the short post-elongation pause and the part of the contraction before the posterior sucker is released; Baader and Kristan, 1992); a contraction phase, following the release of the posterior sucker; and a post-contraction pause, when the two suckers are again attached but the distance separating them is greatly reduced (Stern-Tomlinson *et al.* 1986). The elongation phases of inchworm (e.g. Fig. 2 frames 3–12) and vermiform (data not shown) crawling are similar kinematically and produced sharp increases in pressure which peaked at 11.02±0.54 cmH_2O (89 crawling steps pooled from six animals).

The peak in the pressure correlated closely with the maximum elongation of the animal and the attachment of the anterior sucker (Fig. 2, frame 12). To study the relationship between maximum elongation and peak pressure more closely, we measured the pressure between segments 6 and 8, along with the length of three anterior portions of the body during 11 crawling steps pooled from two animals. The three anterior portions were: anterior sucker to segment 4, segments 4–6 and segments 6–8 (Fig. 3A). Both pressure and length measurements were normalised between maximum and minimum values so that data from different crawling steps could be combined. Fig. 3B shows normalised data from one complete step. The pressure started to increase only near the end of elongation. Different crawls were aligned using the peak pressure (Fig. 3C). On average, the three anterior portions of the body did not reach maximum elongation at the same time [repeated-measures ANOVA: $F(2,10)=14.538$; $P<0.01$]; rather, the maximum elongation of the anterior sucker to segment 4 preceded that of segments 4–6 by 0.51±0.12 s. This supports a front-to-back progression of elongation as has been shown previously (Stern-Tomlinson *et al.* 1986). However, we could not distinguish statistically (Tukey–Kramer multiple comparison $q=1.92$, $P>0.05$, $N=11$) any time difference between the maximum elongation of segments 4–6 and that of segments 6–8, suggesting that the front-to-back progression of elongation, at least as measured by maximum length, may not follow a strict segment-by-segment sequence.

The pressure peak occurred at the same time as the maximum elongation of segments 4–6 [one-sample *t*-test: $t(10)=1.10$,

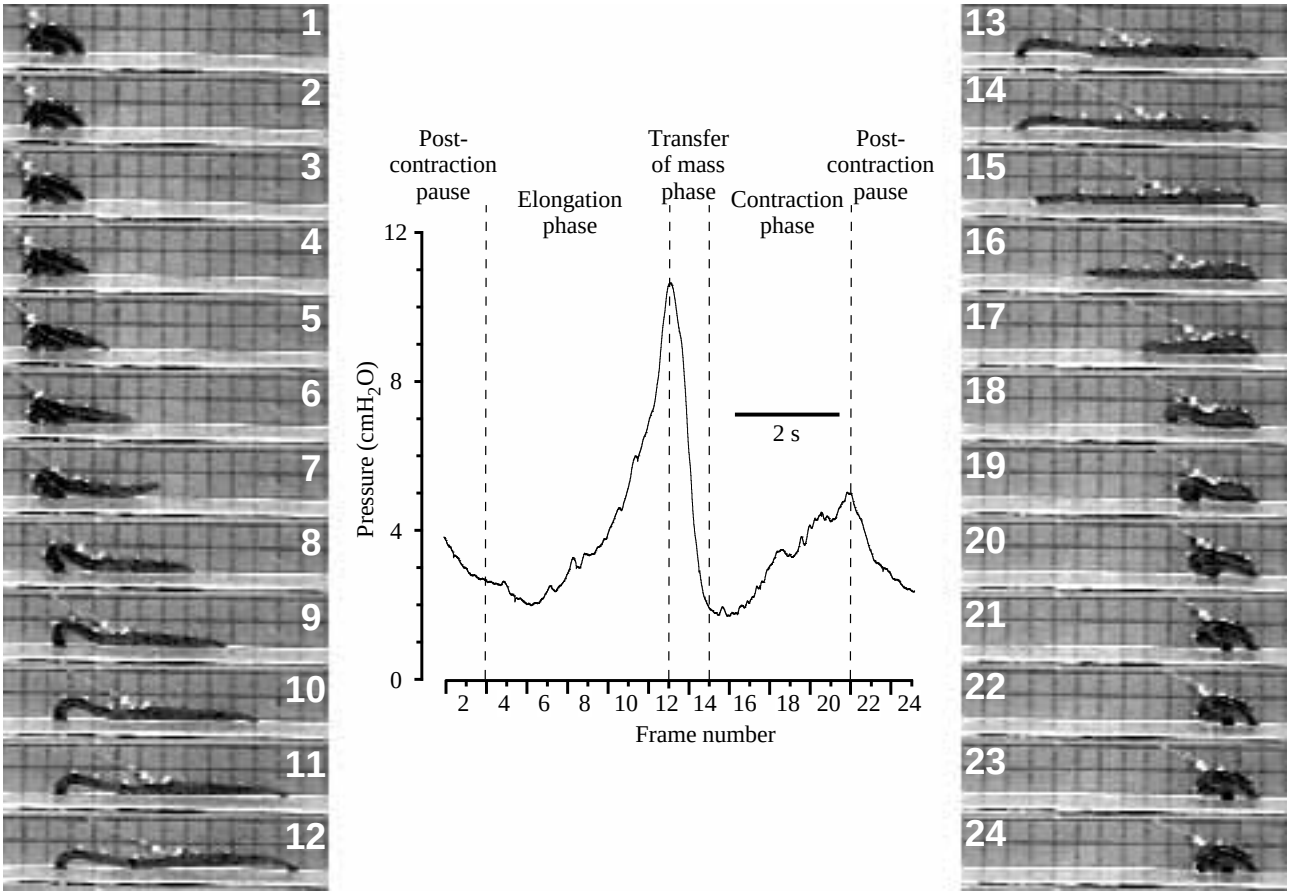


Fig. 2. Pressure measured during inchworm crawling. Frames show a complete step at 0.4 s intervals. Frames 1–3: post-contraction pause. Frames 3–12: elongation phase. Frames 12–14: transfer of mass phase. Frames 14–21: contraction phase. Frames 21–24: post-contraction pause. The trace shows the corresponding pressure. Note that the largest peak occurred at the end of the elongation phase (frame 12) and the smaller peak occurred at the end of the contraction phase (frame 21). 1 $cmH_2O=0.1\ mN\ mm^{-2}$.

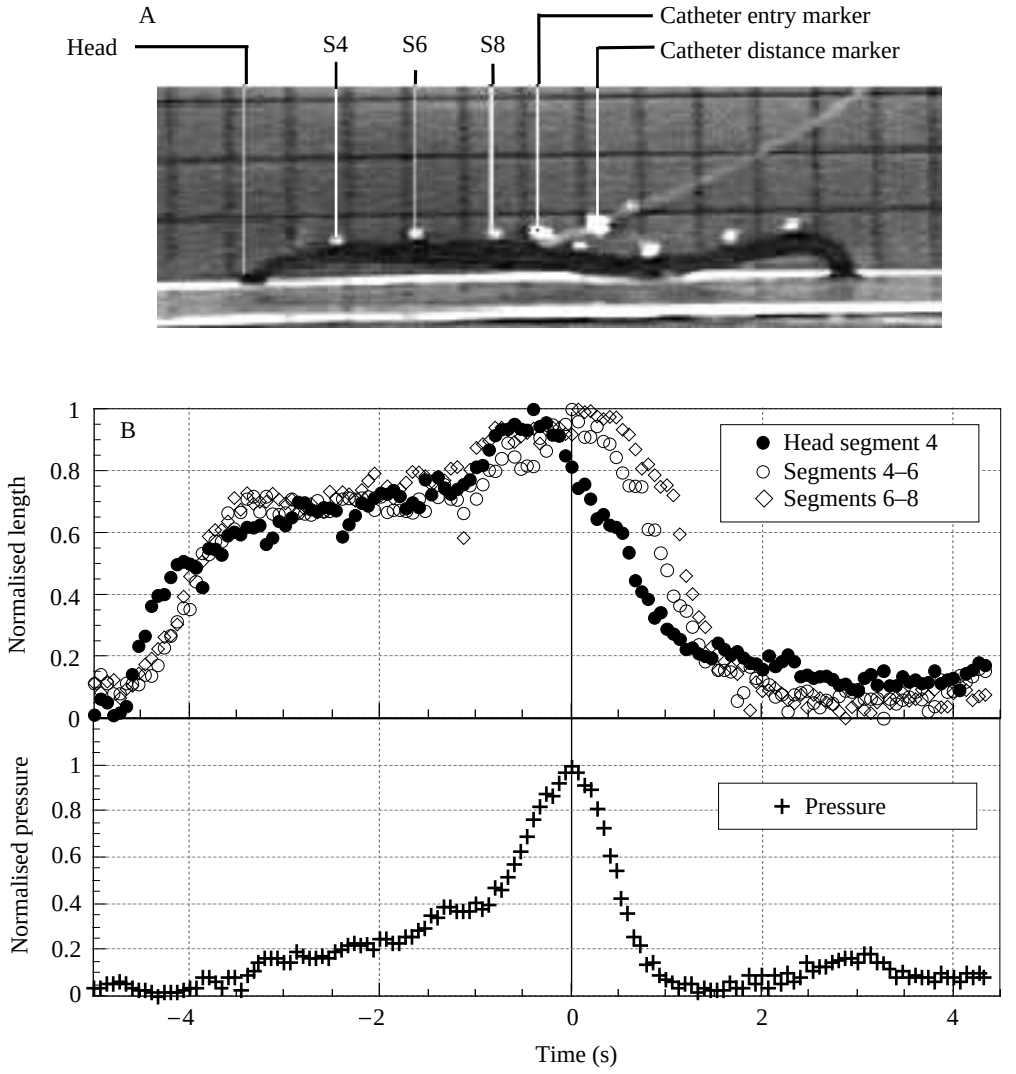


Fig. 3. Pressure as a function of length during crawling. (A) Method of quantification. NIHimage software was used to measure the lengths between the anterior sucker and segment 4 (S4), segments 4–6 and segments 6–8 of leeches filmed during crawling. The distance separating the two white beads on the catheter was used to extrapolate the position of the end of the tube within the animal (when passed through the body wall of segment 9, the tip within the gut was typically located between segments 6 and 8). (B) Normalised length and pressure data from a single, complete step with the corresponding pressure waveform. (C) Averaged normalised data from 11 crawls, aligned to the pressure peak. Values are means ± S.E.M.

P=0.29] and segments 6–8 (one-sample t-test: $t(10)$=1.27, P=0.23]. Since the elongation of the anterior sucker to segment 4 precedes that in the more posterior segments, the peak in pressure occurred *after* the front portion was maximally elongated [one-sample t-test: $t(10)$=4.90, P<0.01]. In fact, by the time the peak in pressure had occurred, the anterior sucker had attached and the front portion of the animal had already shortened to 74.2±3.5 % (N=11) of its maximum length [one-sample t-test: $t(10)$=7.27, P<0.01]. Thus, the pressure recorded in the gut between segments 6 and 8 appeared to reflect movements locally rather than movements in more rostral segments.

It should be noted that shortening of the most rostral segments need not imply a reduction in the total length of the animal, since prior to rostral shortening the anterior sucker attaches and the two suckers then anchor the overall length of the animal. With the rostral segments shortening while the caudal segments continue to elongate, the centre of mass moves towards the rostral end; then the posterior sucker releases and the contraction phase begins. During this transfer of mass, which lasted approximately 1 s, the pressure fell dramatically (Fig. 2, frames 12–14; Fig. 3B,C) to 22.5±2.6 % (N=89) of the peak value. In fact, we found no significant difference between the pressure following the transfer of mass and the baseline pressure measured before the elongation phase (Tukey–Kramer multiple-comparison test: q=2.3048, P>0.05, N=89). If the transfer of mass towards the anterior sucker occurred chiefly because of strong active contraction of the longitudinal muscle acting against the force produced by the circular muscles, one would expect the pressure to increase as a result of co-activation. No such pressure increase was observed. A more likely explanation for the shifting of the centre of mass, therefore, was the release of stretched longitudinal elements, which 'spring back' towards their resting length as the circular muscles relax. During the transfer of mass, the segmental length fell at a rate of about 20 % s^{-1} (Fig. 3C). This was followed by a much faster fall in segmental length (60 % s^{-1}) which began as the pressure levelled out. The rapid change in length may have been caused by the release of the posterior sucker (Fig. 2 between frames 14 and 15), which defines the beginning of the contraction phase.

The pressure profile seen during the later stages of the contraction phase of crawling depended on the type of crawl. During inchworm crawling, contraction produced a shortening of the front of the body and a bend in the rear of the body, with the posterior sucker being placed close to the anterior sucker (e.g. Fig. 2, frames 15–21). This probably requires asymmetric longitudinal and/or circular muscle activation. Dorso-ventral muscles in the rear of the animal, which are attached to the ventral body wall in one segment and extend to the dorsal body wall of the more rostral neighbouring segment, may also play a role (W. B. Kristan and R. J. A. Wilson, unpublished observations). The anterior segments (at least to segment 8) did not appear to be directly responsible for forming the loop, since their length decreased monotonically during the contraction phase (Fig. 3B). However, the pressure between segments 6 and 8 increased significantly during this phase (Tukey–Kramer multiple-comparison test: q=8.15, P<0.001, N=89), peaking as the posterior sucker was attached at 42±2.5 % (N=89) of the peak pressure recorded during elongation. This second peak was probably due to active contraction of the body induced by longitudinal motor neurones in anterior segments. The subsequent fall in pressure occurred during the post-contraction pause (e.g. Fig. 2, frames 1–3 and 21–24). This fall in pressure appeared to be correlated with a loss of tonus which caused the looped body to 'slump' (e.g. Fig. 2, frames 1–3 and 21–24). In vermiform crawling, the contraction phase consists of the animal shortening along its longitudinal axis with its body flat against the substratum (Stern-Tomlinson *et al.* 1986). In the few examples we recorded, we saw no secondary pressure peak; instead, the pressure decreased monotonically from the peak pressure observed during elongation.

Single-site pressure recordings during swimming

Leech swimming comprises dorsal–ventral undulations of a flattened, elongated body (Kristan *et al.* 1974). In some episodes of swimming, the animal first elongated and then began to undulate; in others, the undulations occurred as the animal elongated (Fig. 4, frames 1–11). In both cases, elongation caused an overall increase in pressure, but the undulating 'take off' had a more jagged pressure profile. Once the posterior sucker had released and the animal began to make progress through the water (e.g. Fig. 4, frames 11–20), the elevated pressure was maintained at about twice the maximum value recorded during crawling. The elevated pressure involved phasic fluctuations of between 20.9±1.2 and 26.9±1.5 cmH_2O (N=49 swims, pooled from six animals).

To relate the phasic fluctuations in pressure with the phase of the swim, we measured the pressure between segments 6 and 8 as well as the phase of the body undulation at the location of the end of the catheter (Fig. 5). The pressure was normalised between the lowest and highest pressures measured in any one swim so that data from different swims and different animals could be combined. Swim phase has been defined with 0 ° corresponding to the middle of the period of activity of the dorsal longitudinal motor neurones (e.g. Stent *et al.* 1978). Because we did not measure motor neurone activity, we defined 0 ° phase in a given segment as the time when the pressure trough (see Fig. 5) was centred on that segment, which corresponds to maximal contraction of the dorsal longitudinal muscles. Fig. 5B shows pooled data for 17 swims obtained from a single leech. For this leech, each swim cycle had two peaks in the pressure recording. One pressure peak occurred when the segment in which the end of the tube was located was half-way through an upward swing (90 °). The other peak occurred when the estimated position of the end of the tube coincided with the middle of a downward swing (270 °). Although two pressure peaks per swim cycle was a common feature of our recordings, the relative sizes of the peaks and troughs varied from cycle to cycle (Fig. 4), from swim to swim (Fig. 5B) and from animal to animal (Fig. 5C). Fig. 5C shows measurements from 538 frames of 61 swims from four leeches pooled in bins of 20 °. The fluctuation in pressure with swim phase was statistically significant [ANOVA of bins: $F(17, 521)$=3.83, P<0.001].

However, although the data suggest two pressure peaks per cycle, the apparent low point centred at 0 ° was not statistically significantly different from the pressure recorded between either 270 and 290 ° (Boneferroni multiple-comparison test: t=2.21, P>0.05) or 70 and 90 ° (Boneferroni multiple-comparison test: t=1.35, P>0.05). The other low point in pressure at 170–190 ° was more robust, the pressure during this phase being significantly smaller than that at 70–90 ° (Boneferroni multiple-comparison test: t=3.59, P<0.01) and 250–270 ° (Boneferroni multiple-comparison test: t=3.58, P<0.01). Thus, on average, the most significant fall in pressure occurred at the transition from the recovery stroke to the power stroke.

Single-site pressure recordings during shortening

In active animals, head pokes often produce a rapid whole-body shortening response caused by the co-contraction of dorsal and ventral longitudinal muscles in many body segments. This behavioural response habituates rapidly and frequently includes a curl that tucks the head against the ventral surface of the body. When the pressure generated during shortening was most sizable, the pressure profile often had two peaks (e.g. Fig. 6). The first peak (9.7±1.5 cmH_2O, N=20) was the largest (Wilcoxon signed-rank: Z=−2.35, P<0.05). No detailed comparison between the kinematics of shortening and pressure was performed, since both the kinematics and the pressure profile were too variable. However, it did appear that the first peak corresponds to the actual shortening, whereas the second peak may be correlated with reorientation for subsequent movements. We found that the first peak was similar in magnitude to the pressure peak during the elongation phase of crawling, but was significantly larger than the peak during the contraction phase (Dunn's multiple comparison test: P<0.01).

In summary, crawling, swimming and shortening produced distinctive pressure waveforms. During crawling, the pressure appeared to be localised to discrete portions of the body, because peak pressure was best correlated to local elongation. This suggests that the body is compartmentalised. During swimming, the pressure waveform was a function of phase. This can also be interpreted in a framework in which the body is compartmentalised and the pressure is a function of the local

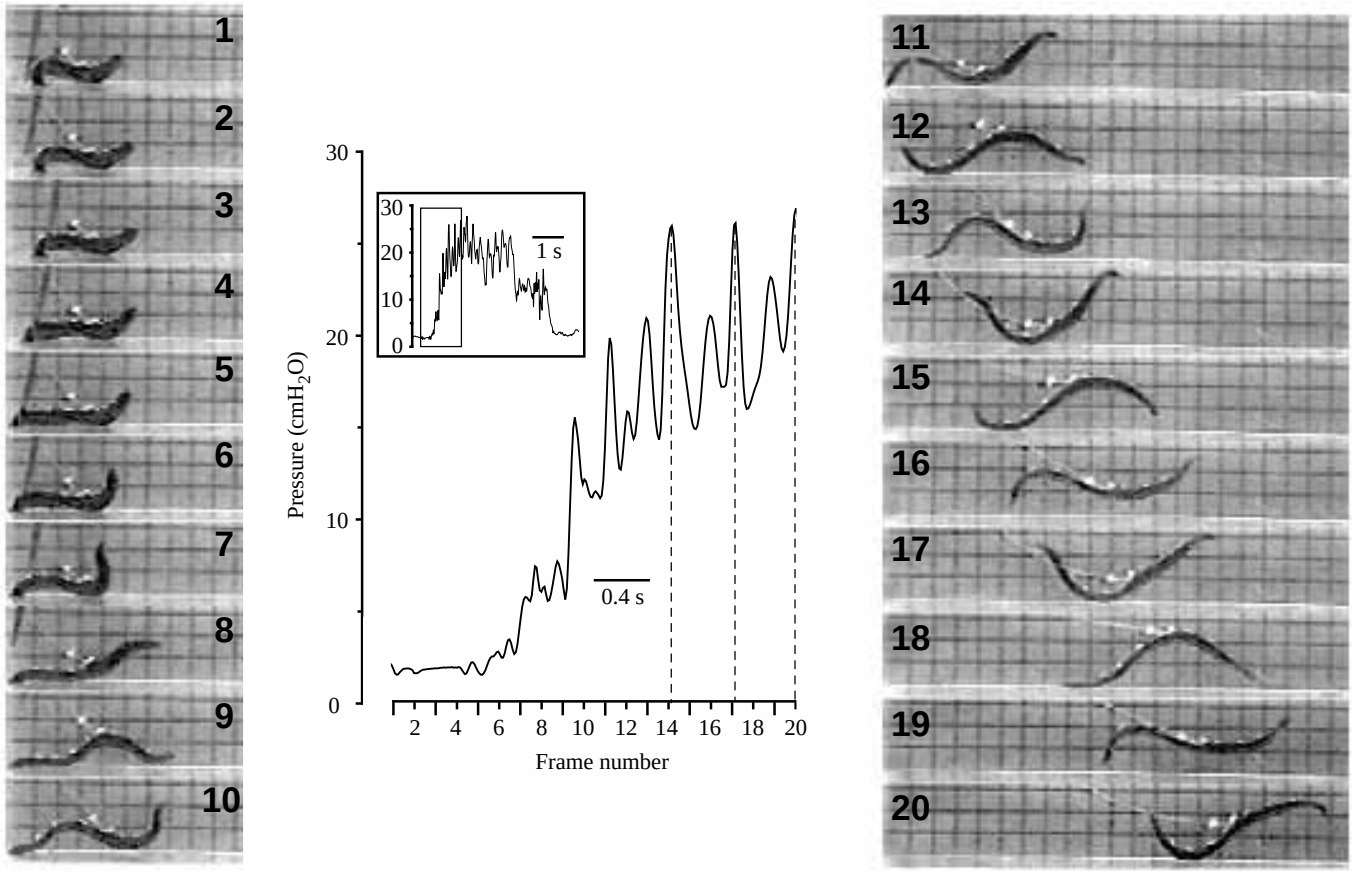


Fig. 4. Pressure during swimming. Frames taken at 0.133 s intervals showing the beginning of a swimming episode. The catheter entered the leech through segment 9. The distance separating the two white beads on the catheter was used to determine the position of the end of the tube within the animal (typically between segments 6 and 8). Frames 1–11: take-off phase of swimming evoked by a tail poke. Frames 12–18: two complete cycles of swimming. The trace shows the corresponding pressure (the inset shows the pressure for the whole swimming episode). Swimming produces high pressure which fluctuates rhythmically. Note that, between frames 12 and 18, there were four peaks, i.e. two peaks per cycle. The peaks occurred at phases when the curvature of the body at the tip of the tube was minimal (dashed lines). 1 cmH_2O=0.1 mN mm^{-2}.

muscle contraction. However, it does not rule out the possibility that pressure is uniform along the whole leech and is a function of the shape of the whole animal. To address this question more directly, we measured the pressure at two locations in active animals.

Dual-tube recordings

Implanting two tubes into the gut doubled the mass that the leech had to pull and tended to distort body movements. To overcome this problem, we used large leeches (>3 g), inserted the tubes through the body wall only four segments apart (segments 9 and 13) and quantified examples of behavioural patterns from the four leeches whose movements were least distorted. The pressure waveforms closely resembled those obtained with a single tube (e.g. Fig. 7A).

Although the pressure recorded from the two locations shared the same characteristics, they were never identical (asterisks in Fig. 7Ai,Bi,Ci). The peaks differed in rate of rise

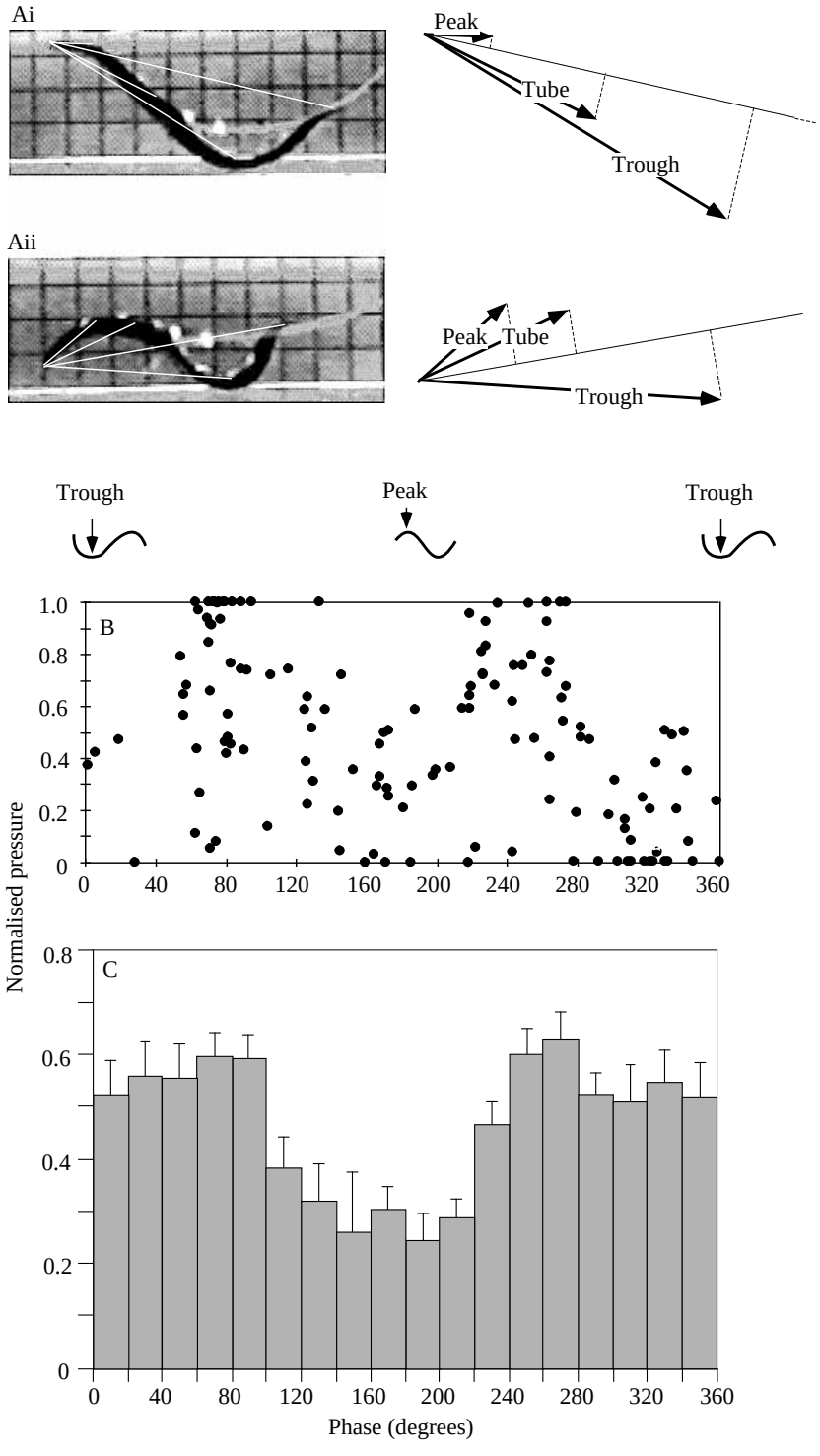
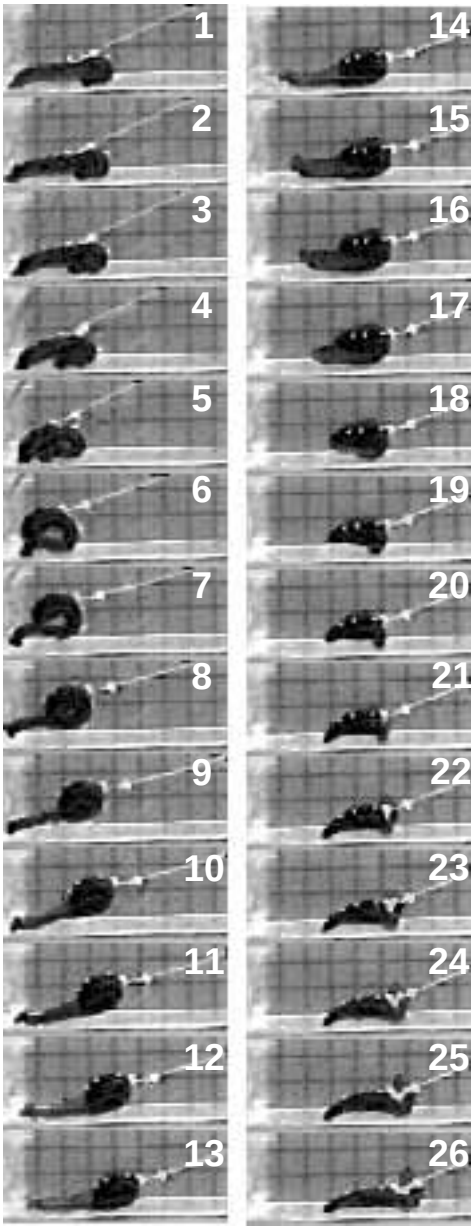


Fig. 5. Fluctuations in pressure as a function of phase during swimming. (Ai,ii) Estimation of the tip location of the pressure-measuring tube relative to the peaks and troughs of the body waves in swimming leeches. NIHimage software was used for the analysis. For each frame, a line was drawn from the anterior to the posterior sucker as an axis to correct for pitch. Lines were then drawn from the anterior sucker to the estimated location of the tip of the tube and to the points were the body wall was farthest from the axis, i.e. one above the axis (peak) and one below the axis (trough), making a total of three additional lines per frame. The angles (relative to the pitch axis) and the lengths of the three additional lines were measured. The relative axial distance of the tube tip location between the peak and trough was calculated. This relative axial distance was then converted to phase such that when at 0° phase the tip of the tube was in the middle of a trough and when at 90° phase the tip of the tube was in the middle of an upward swing. In Ai, phase at the tip of the tube is close to 90° whereas in Aii, when the tube is nearer the peak than the trough, the phase is between 90 and 180°. (B) Pressure *versus* phase for one leech. For any one swimming episode, the pressure was normalised between the lowest and highest values so that different episodes could be combined (139 frames from 17 swimming episodes). Note that there are two low points and two high points in the pressure trace. The high points corresponding to phases of low curvature, i.e. in the middle of the upward and downward swings. (C) Data from four animals (538 frames from 61 swimming episodes). The relative magnitudes of the fluctuations in pressure varied from swim to swim and from animal to animal. The graph shows that the relatively low pressure at 180° (when the tube is at a peak) is the most resilient feature. Values are means + S.E.M.



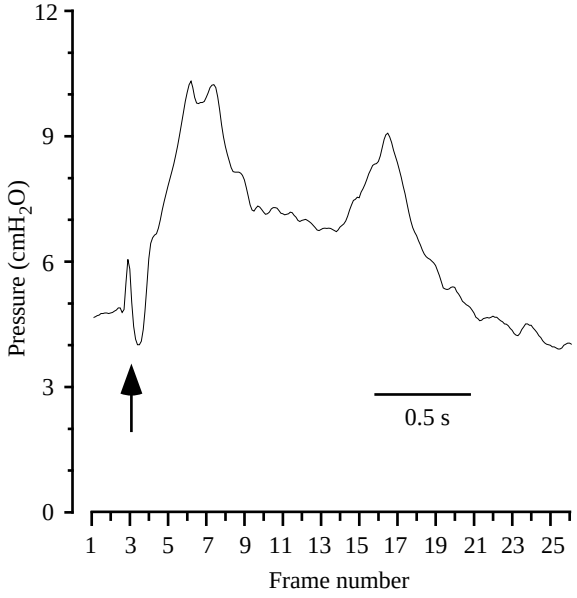


Fig. 6. Pressure following a head poke. In freely behaving leeches, a head poke produces a whole-body shortening reflex which is variable in nature, making this behaviour difficult to quantify kinematically. Frames 1–26 show an example of a response to a head poke delivered during the contraction phase of crawling. After a head poke was delivered at frame 3 using a pointed stick, the pressure increased rapidly following a brief artefact (arrow). Between frames 5 and 11, the animal curled, shortening its rostral segments and elongating its caudal segments, replacing its posterior sucker farther to the left. The pressure peak occurred when the animal was curled most fully (frames 6–8), falling even though the elongation in caudal segments continued (frames 8–13). The second peak in pressure corresponds to when the leech begins to regain its posture on the way to attaching its anterior sucker (frame 17). Two pressure peaks were a common occurrence after a head poke, but other waveforms were also observed (e.g. see Fig. 7). $1\,cmH_2O=0.1\,mN\,mm^{-2}$.

and in magnitude, but the difference was not consistent between the two recording sites. Differences in pressure recorded at two sites simultaneously may have arisen as a consequence of subtle changes in the pattern or degree of muscle activation between the two locations from one episode of a movement to another. It is also possible that the differences arose as a result of the catheters blocking slightly, but since the ends of the tubes were funnelled and the shafts were perforated immediately adjacent to the ends, this possibility seems unlikely.

We found no evidence for a consistent delay in the pressure peaks that occurred during shortening [Fig. 7Cii,iii; one-sample *t*-test: $t(30)=1.18$, $P=0.24$]. During crawling, the pressure peak in segment 9 preceded that in segment 13 by 45±24 ms, but this difference was not quite significant [(Fig. 7Aii,iii; one-sample *t*-test: $t(52)=1.91$, $P=0.06$]. These results suggest that, during these types of behaviour, any four segments can be thought of as a single compartment.

During swimming, there was a consistent delay between the pressure recorded in the two locations [Fig. 7Bii,iii; one-sample *t*-test: $t(41)=4.66$, $P<0.0001$). The pressure peak recorded between segments 6 and 8 nearly always preceded that recorded between segments 10 and 12. On average the delay was 37±8 ms. This is within the bounds of the intersegmental travel time for dorsal–ventral undulations observed in previous kinematic studies (Kristan *et al.* 1974).

Effects on pressure of injecting fluid into the gut

A key feature of the lifestyle of the leech is its blood-sucking activities. When *Hirudo medicinalis* feed they can consume almost nine times their body mass in a single meal (Lent *et al.* 1988). This translates into a huge increase in gut volume which

must have a profound effects on the biomechanics of the system. To examine these effects, we recorded pressure in leeches as they fed on a blood-filled sausage casing. As the leeches pumped blood into their crop with their pharynx, we recorded a rhythmic pressure waveform that fluctuated by less than 5 cmH_2O around the resting pressure (data not shown). As the volume of the gut increased, we expected a sharp increase in pressure, as a result of the passive length–tension relationships of the surrounding muscles (Wilson *et al.* 1995*b*). Instead, however, the pressure decreased. Since the newly ingested blood may have been blocking the catheter, we performed a series of experiments in which we artificially inflated the gut by injecting water through the catheter in 1 ml increments. We then measured the pressure immediately before a movement, at the first peak, at the first

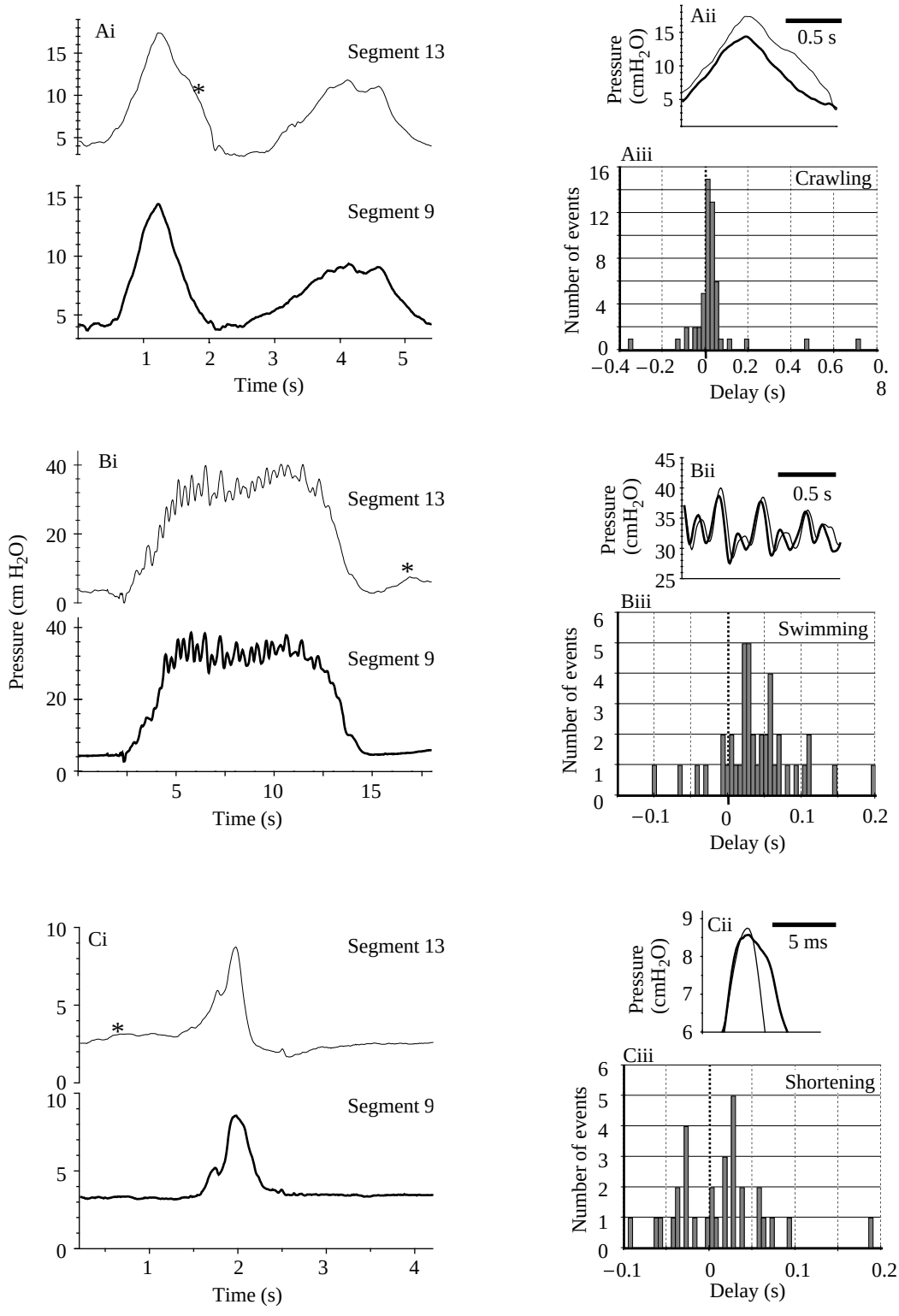


Fig. 7. Compartmentalisation of pressure. (Ai,Bi,Ci) Pressure recorded at two sites during crawling, swimming and shortening, respectively. Tubes were passed through the body wall into the gut at segments 9 and 13, tips were located between segments 6–8 and 10–12 respectively. The pressure waveforms recorded at the two sites were broadly similar, although they frequently differed in fine structure (asterisks). (Aii,Bii,Cii) Portions of recordings overlaid and at higher resolution (bold line, segment 9; light line, segment 13). (Aiii,Biii,Ciii) Histograms showing the delay between pressure peaks at the two locations. Positive values were assigned when the pressure in the rostral location led that in the caudal location. For all three activities studied, there were examples of the pressure in the more caudal location leading that in the more rostal location (negative values). On average, however, the pressure peaks in the rostral location preceded those in the caudal location. 1 cmH_2O=0.1 mN mm^{-2}.

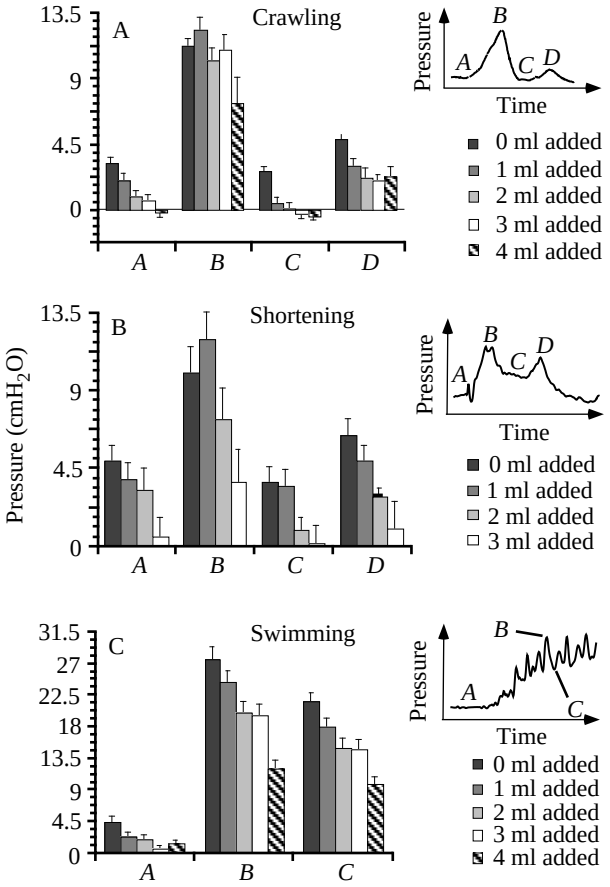


Fig. 8. Pressure *versus* injected volume. (A–C) Pressure measurements during swimming, shortening and crawling at different volumes. To quantify the effects of adding volume, we measured the amplitude of the principal features in the characteristic pressure waveforms during crawling, shortening and swimming, respectively. In all cases, a 'resting' measurement was made immediately before the activity commenced (bars labelled *A*). For crawling (A), we measured the peaks that were correlated with the ends of the elongation phase (bars labelled *B*) and the contraction phase (bars labelled *D*), as well as the intervening trough (bars labelled *C*). Three measurements were also made following head pokes (B) similar to those made for crawling, although only prominent waveforms were considered in order to quantify only the most vigorous shortening responses. For swimming (C), we chose the most salient peak (bars labelled *B*) and trough (bars labelled *C*). 1 cmH_2O=0.1 $mN\,mm^{-2}$.

trough and at the second peak during different activities (bars labelled *A*, *B*, *C* and *D*, respectively, in Fig. 8). In swimming, for which only a single pressure peak and trough were selected, we measured those that were most prominent. Each of the types of behaviour retained its characteristic pressure profile, but as the injected volume increased the amplitudes of the pressure changes decreased [multiple regression analysis using all the measurements across all the types of behaviour: $t(1367)=8.237$, $P<0.0001$]. For a 1 ml increase in volume, the pressure fell by 1.34 cmH_2O (i.e. the regression coefficient for volume was −1.34).

Discussion

Internal pressure and behaviour in the leech and other invertebrates

In this paper, we have quantified the pressure inside the gut of leeches during different types of behaviour. Each behavioural activity has a distinct pressure waveform that differs in both magnitude and profile. The resting pressure of leeches was low, typically less than 4 cmH_2O. Swimming was associated with a high sustained pressure which on average fluctuated phasically between 21 and 27 cmH_2O. The elongation phase of crawling produced an increase in pressure which peaked on average at 11 cmH_2O at the end of elongation and then fell to the resting level irrespective of animal length. The contraction phase of crawling produced a pressure peak only during inchworm crawls, in which the posterior sucker was placed close to the anterior sucker and the middle of the body was lifted off the substratum. Whole-body shortening generated internal pressures of similar magnitude to those produced during the elongation phase of crawling, but the rise times were faster. Behaviourally distinct pressure waveforms have also been reported in other invertebrates. For example, the pressure waveforms during tail withdrawal and burrowing in *Bullia digitalis* (Trueman and Brown, 1976), tail withdrawal and head waving in *Aplysia californica* (Kuenzi and Carew, 1994) and locomotion and burrowing in annelids other than the leech (e.g. Chapman and Newell, 1956; Seymour, 1971) have been described. Interestingly, the pressure profile recorded in earthworms *Lumbricus terrestris* during crawling (Seymour, 1969) is strikingly similar to that recorded in the leech during inchworm crawling, even though the kinematics of the crawls are quite different and the earthworm has tight septa which compartmentalise the body cavity. The magnitudes of pressures recorded in *Lumbricus terrestris* (Seymour, 1971) and *Arenicola marina* (Trueman, 1966) are similar to those reported here.

Internal pressure is a key component of the mechanics of locomotion. For example, the legs of some spiders have an elevator but no protractor muscle (Parry and Brown, 1959). In these animals, the requirement for muscles to be arranged antagonistically is alleviated by the inherent pressure of the body cavity. The pressurised body cavity contributes to rigidity during leg elevation and is responsible for returning the legs to the resting position once the elevator has relaxed. A similar mechanism is used by barnacles and some insect larvae (Cannon, 1947). More commonly, however, internal pressure is generated by the activation of antagonistic muscles, and the balance between the activation of antagonists determines both the movement and the degree of rigidity. The anatomical organisation of antagonistic muscles is diverse, ranging from the limbs of vertebrates, in which muscles are attached about a single joint, to organs that lack hard skeletons, such as tongues, trunks and tentacles, in which the antagonistic muscles are arranged around each other to form a hydroskeleton. Of these hydroskeletal structures, some have muscles which surround a fluid-filled cavity (e.g. worms; Chapman, 1950), whereas others, termed muscular hydrostats, are composed almost entirely of muscles (e.g. tentacles, trunks and tongues; Kier and Smith, 1985). Potentially, leeches might span

these two categories during their lifetime: the body cavity of fed leeches can occupy almost 90 % of the volume of the animal (Lent *et al.* 1988), whereas when leeches are starved their body cavity is practically empty and the hydroskeleton may best be described as a muscular hydrostat.

Compartmentalisation of the body cavity

It is constructive to consider the biomechanics of hydrostats in terms of functional compartments. Using this framework, muscular hydrostats (such as tongues and tentacles) represent an extreme case of compartmentalisation, in which the internal pressure generated by muscle activation is localised to a finite volume about the activated region. Septate worms such as *Lumbricus terrestris* have bigger body compartments, but the internal pressure remains confined to active body segments (Seymour, 1969). This morphology may be useful to earthworms, allowing them to pry open crevices in hard soils (Seymour, 1970). Aseptate worms, such as *Arenicola marina*, represent the other extreme to muscular hydrostats, in which the whole animal can be considered as a single compartment. If one end of *A. marina* elongates, then the resulting pressure is distributed throughout the animal, causing the other end to swell. This worm may make use of this swelling effect to increase overall traction (at the expense of local force) whilst burrowing in the soft medium in which it lives.

The results in this paper strongly suggest that the body cavity of the leech is compartmentalised during at least two types of behaviour, crawling and swimming, even though it lacks the septa that compartmentalise the body cavity of less-specialised worms such as *Lumbricus terrestris*. We demonstrated that, during crawling, the pressure peak recorded using a single catheter correlated best with local elongation (i.e. recordings made between segments 6 and 8 were independent of changes in length in the most rostral segment; Fig. 3B,C). In contrast, dual-tube pressure recordings, in which the tubes were separated by four segments, demonstrated a pressure delay that was not quite significant (Fig. 7Aiii). Furthermore, we could not distinguish statistically segments 4–6 from segments 6–8 in terms of their time course of elongation, suggesting that a functional compartment during crawling may span as many as four segments (Fig. 3B,C). During swimming, the fluctuations in the sustained pressure plateau oscillated in phase with local movements, also suggesting a local, rather than a global, activity-dependence. Dual-tube recordings demonstrated that the pressure waveforms during swimming were indeed local and that the delay in the pressure recorded at the two sites could be accounted for by the intersegmental delay in activation.

Functional compartmentalisation is supported by the observation that elongation in the rostral segments during crawling does not cause the caudal segments to swell, even in fully fed leeches (R. J. A. Wilson, unpublished observation). Results from modelling studies also support compartmentalisation, since an abstract uncompartmentalised model of the leech began to capture the kinematics of behaviour only when compartments were added (Wadepuhl and Beyn, 1989; W.-J. Beyn, C. Alscher and W. B. Kristan, unpublished observations). In the more realistic model we have constructed, we also find compartmentalisation to be important in producing life-like behaviour (B. A. Skierczynski, R. J. A. Wilson, W. B. Kristan and R. Skalak, in preparation).

How then is the body compartmentalised? Although the leech lacks segmental septa, the gut does contain sphincters at segment boundaries which could compartmentalise the body cavity. It should be noted that the wall of the gut is very elastic so that, although the sphincters may prevent bulk flow over long distances, they may not prevent the volume of one segment partially displacing that in the next. Following a large blood-meal, this problem may be alleviated somewhat by the increased gut wall stiffness that results from stretch. Another mechanism by which a blood-meal may be compartmentalised is by the bundles of dorso-ventral muscles which traverse the body cavity. Interestingly, the compartmentalisation is most obvious during swimming, when these muscles are activated (Ort *et al.* 1974). Activation of the dorso-ventral muscles flattens the animal and is likely to increase its stiffness. These muscular pillars are likely to resist the displacement of volume from one segment to another most effectively if the bulk is viscous. The urinary bladders can contain a large proportion of the fluid in the body cavity (Wenning *et al.* 1980), and these would also restrict the fluid to their own segment. It is also enticing to note that leeches increase the cell concentration of digested blood by excreting plasma both during and immediately after feeding. This may act to increase the protein content, and therefore the energy value, of the gut contents but it also increases their viscosity.

Peak pressure during crawling and the strength of the circular muscles

There are no stiff anatomical structures separating the muscles, which generate the force, from the inside of the body cavity or the inside of the gut. Therefore, when the muscles in the body wall contract, they are likely to pressurize the entire body cavity. Thus, the magnitude of the pressures within the gut during different types of behaviour (Fig. 8) allows a rough prediction of the forces that the muscles must generate (Chapman, 1950; Seymour, 1969). During the elongation phase of crawling, the pressure in a segment increases monotonically until the maximum length is achieved. The peak pressure of 11 cmH_2O, or 1.1 $mN\,mm^{-2}$, results from the interaction between the force produced by the activation of the circular muscles, A_c, which are responsible for elongation, and the passive tension in the longitudinal muscles, F_l, which resist stretch. For convenience, we shall combine the inherent activation of longitudinal muscles with the passive tension of the connective tissue (Wilson *et al.* 1995*b*). We have established previously that the body wall generates a passive, resistive force of 38 mN at its maximum length (Wilson *et al.* 1995*b*). If segments are assumed to be compartmentalised and of fixed volume with an idealised circular cross section, then the internal pressure, P, required to generate such a force can be estimated from the radius, r, of a maximally elongated segment (Chapman, 1950):

$$P = F_l/(\pi r^2). \quad (1)$$

Using an estimated radius of 2.2 mm (Wilson *et al.* 1995*b*) gives a value of 2.49 mN mm^{-2}. This idealised value is within the same order of magnitude as the pressure measured experimentally (1.1 mN mm^{-2}). Thus, we can use the experimental value to estimate the order of magnitude of the active force delivered by the circular muscles, A_c. From Chapman (1950):

$$A_c = Prl . \quad (2)$$

Using a value of 4 mm for the length, *l*, of a maximally elongated segment (Wilson *et al.* 1995*b*) gives an estimated force of 9.68 mN. This is the force that the circular muscles in each segment must *deliver* (when they are at their shortest length) to generate the internal pressure and equilibrate the longitudinal passive force when the leech is maximally elongated. It is not the total force that the circular muscle must *produce* for it must also provide a force to compress the circumferential axis. Since the circular muscles in neighbouring segments act in parallel, the summed force-delivering capability of the circular muscles is 20 times this value, approximately 194 mN. This is comparable to the force that the longitudinal muscles must generate when shortened, given the force they can generate when at optimal length (600 mN) and their bell-shaped length–tension curve (Wilson *et al.* 1995*a*). Since the circular and longitudinal muscles have similar total cross-sectional areas and should, therefore, be able to generate the same force, 194 mN may be a reasonable estimate of the force required for elongation.

Peak pressure during swimming and the strength of the dorso-ventral muscles

Leech swimming involves a flattening of the body and dorsal–ventral undulations. The flattening is produced in part by the dorso-ventral muscles, which are activated during swimming episodes (Ort *et al.* 1974). Although some of these muscles are located within the lateral edge of the body wall, most span the body cavity and are accessible for experimentation. We have measured the force produced by this subset of muscles during swimming in a single segment of a semi-intact leech and have found that they can generate up to 120 mN (R. J. A. Wilson, unpublished data). However, since the pressure during swimming can be as high as 26 cmH_2O, or 2.6 mN mm^{-2}, the force required to prevent the cross section from adopting a round profile is relatively large (218 mN, based on an estimated horizontal cross section of 84 mm^2 per segment). Thus, it would appear that the dorso-ventral muscles are not sufficient to maintain the leech in a flattened state, and an additional explanation is required. One possibility is that the body cavity within each segment is divided into chambers, such that the pressure generated in each chamber acts on a smaller horizontal cross section and thus is more easily opposed. The dorso-ventral muscles that span the body cavity may provide compartmentalisation by partially isolating the gut mechanically from the two side pouches associated with it. Another possibility is that the dorsal–ventral undulations are responsible. If a stiff tube is bent, it will flatten around the point of the bend. Similarly, the body of a swimming leech is relatively stiff (leeches will 'swim' in air) and the local longitudinal curvature that results from the shortening of the dorsal or ventral side of the body wall may have a flattening effect whilst also generating internal pressure. A third possibility is that circular muscles, which have been shown to be active phasically during swimming (Baader and Kristan, 1992; J. Eisenhart, unpublished data), contribute to the internal pressure and flattened shape. Preliminary results from studies using our mechanical leech model suggest that circular muscles are necessary to generate swim-type leech shapes (B. A. Skierczynski, unpublished results).

Peak pressure decreases as the volume increases

When the volume of the gut of the leech was increased by injection of water, we found that pressure during specific activities remained characteristic of that activity, but that the amplitude of the pressure decreased (Fig. 8). Similarly, when leeches fed, the increase in volume did not produce an increase in pressure. Interestingly, Wadepuhl and Beyn (1989) constructed an abstract leech model based on linear length–tension curves which predicted that increasing volume caused a reduction in pressure. They attributed this effect to Laplace's law, which states that the pressure in a body decreases with increasing volume providing that the surface tension remains constant. We have since measured the length–tension properties of the longitudinal and circumferential axes of the leech body and have found that the tension is approximately proportional to the cube of stretch, such that at low volume the passive tension is slight whereas at high volume the passive tension is sizable (Wilson *et al.* 1995*b*; see also Miller, 1975). Thus, it might be expected that increases in volume beyond a certain point would produce huge increases in passive tension that more than compensate for the Laplace effect of increasing volume and, hence, lead to increases in pressure.

The biomechanical properties are likely to be augmented by serotonin released by the central nervous system. Serotonin increases the frequency of types of behaviour associated with appetitive aspects of leech feeding (e.g. Willard, 1981; Lent *et al.* 1989; Groome *et al.* 1993) and causes a reduction in the passive tension of the body wall in leeches (e.g. Mason and Kristan, 1982; Wilson *et al.* 1995*b*) as well as in blood-sucking insects (Orchard *et al.* 1988). Thus, before feeding, serotonin may reduce the passive tension of the body wall, allowing a greater volume to be ingested without an increase in pressure.

Internal pressure is the product of many different biomechanical factors, including morphology, passive and active steady-state length–tension curves, the pattern of activation and dynamic properties such as resistance to stress, stress relaxation, force–velocity curves and interactions with the environment. Biomechanical models will help us to understand how these many factors interact to produce overt behaviour (Chiel *et al.* 1992; Ekeberg, 1993). The present study provides data that constitute an important test of present and future biomechanical models of the leech hydroskeleton (Wadepuhl and Beyn, 1989; Wilson *et al.* 1995*a*; B. A. Skierczynski, R. J. A. Wilson, W. B. Kristan and R. Skalak, in preparation).

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