

FUNCTION OF THE INTERCOSTAL MUSCLES IN TROTTING DOGS: VENTILATION OR LOCOMOTION?

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Summary

Although the intercostal muscles play an important role in lung ventilation, observations from fishes and ectothermic tetrapods suggest that their primary function may be locomotion. To provide a broader understanding of the role these muscles play in locomotion, I measured ventilatory airflow at the mouth and activity of the fourth and ninth intercostal muscles in four dogs trotting on a treadmill. During rest and thermoregulatory panting, activity of the intercostal muscles was associated with inspiratory and expiratory airflow. However, during trotting, activity of the interosseous portions of the intercostal muscles was correlated with locomotion. When ventilation and stride cycles were not synchronized, activity

of the interosseous intercostal muscles stayed locked to the locomotor events and drifted in time relative to ventilation. In contrast, activity of the parasternal portion of the internal intercostal muscles was always associated with inspiratory airflow. These observations suggest that, in dogs, locomotion is the dominant function of the interosseous portions of the intercostal muscles. However, the parasternal intercostal muscles are primarily inspiratory in function.

Key words: locomotion, lung ventilation, intercostal muscles, parasternal muscles, locomotor–ventilatory coupling, locomotor stamina.

Introduction

The intercostal muscles have long been known to play an important role in lung ventilation (Hamberger, 1727; De Troyer and Loring, 1986). However, three lines of evidence suggest that locomotion, not ventilation, may be the primary function of these muscles. First, breathing was not the original function of the muscles of the body wall (i.e. the hypaxial muscles). All bony fishes have distinct hypaxial layers very similar in organization to the oblique and rectus abdominis muscles of tetrapods (Maurer, 1913). Because the fish ancestors of tetrapods are known to have used a buccal pump to fill their lungs (Liem, 1985; Brainerd *et al.* 1993), we can be confident that the basic organization of the hypaxial muscles evolved before they became associated with lung ventilation. Consequently, in fishes, the apparent function of the precaudal hypaxial muscles is locomotion.

Another reason to suspect that lung ventilation may not be the principal function of the intercostal muscles is the uncertain mechanical role they play in breathing. The most widely accepted hypothesis is based on the different moments that the two intercostal muscles impose on the ribs and is over 260 years old (Hamberger, 1727; see De Troyer *et al.* 1985). Owing to the craniodorsal orientation of the external intercostal muscles, the caudal insertions have longer moment arms

around the center of rotation of the ribs than the cranial insertions. Thus, when these muscles contract, the moments tending to move the ribs cranially are greater than those tending to rotate the ribs caudally. In contrast, the caudodorsal orientation of the internal intercostal muscles results in larger moments tending to rotate the ribs caudally. These observations led Hamberger (1727) to suggest that the external intercostal muscles should increase thoracic volume and be inspiratory in function, whereas the internal intercostal muscles should act to deflate the thorax. This appealing hypothesis provides a functional explanation for the structural organization of the intercostal muscles. However, an alternative hypothesis suggests that it is the relative resistance of the ribs to cranial and caudal displacement that determines intercostal muscle action, not the orientation of the muscles (De Troyer *et al.* 1985; Carrier, 1989). This idea suggests that the intercostal and associated muscles in the cranial portion of the thorax result in greater resistance to caudal displacement of the ribs. Consequently, both the external and internal intercostal muscles in the more cranial segments should tend to move the ribs cranially when they contract. The converse should be true of the intercostal muscles in the caudal portion of the thorax. Thus, the traditional hypothesis predicts that the

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external and internal intercostal muscles will act as antagonists to produce ventilation, whereas the resistance hypothesis suggests that they will behave as agonists. Although most electromyographic studies (Bronk and Ferguson, 1935; Taylor, 1960; De Troyer and Ninane, 1986; De Troyer and Farkas, 1989) and finite element analysis (Loring and Woodbridge, 1991; Loring, 1992) support the older hypothesis, several electromyographic studies indicate that the external and internal intercostal muscles often act as agonists to produce inspiration or expiration (Gesell, 1936; Kadono *et al.* 1963; Fedde *et al.* 1964a,b; Carrier, 1989). If both muscles can produce either inspiration or expiration, then the traditional view of their ventilatory function, based on fiber orientation, does not provide an explanation for the structural organization of these muscles.

A third reason to view the intercostal muscles as locomotor muscles comes from observations on walking and running lizards. In the lizard *Iguana iguana*, the intercostal muscles function in both lung ventilation and locomotion (Carrier, 1989, 1990, 1991). The cranial 3–4 segments of the external and internal intercostal muscles are the principal effectors of inspiration. Both muscles are activated simultaneously on the two sides of the body to draw the ribs cranially and laterally, producing an increase in thoracic volume. Neither muscle is involved in expiration. Nonetheless, during walking, the intercostal muscles cease to be correlated with ventilation and display a pattern of unilateral activation that appears to be associated with stabilization of the trunk against long-axis torsion.

These observations indicate that the original function of the hypaxial muscles was not lung ventilation and suggest that the current organization of the intercostal muscles may reflect the demands of locomotion. To provide a broader phylogenetic understanding of the role that the intercostal muscles play in locomotion, I monitored muscle activity and ventilatory airflow in four dogs while standing and trotting on a motorized treadmill.

Materials and methods

Electrical activity of the intercostal muscles was monitored in four female dogs (*Canis familiaris*) of mixed breed as they stood and ran at moderate trotting speeds on a motorized treadmill. The four dogs were part of a larger group of seven used to study the activity of the axial muscles during locomotion. Their mean body mass was 18.7 ± 1.7 kg (S.D.). Each was purchased at an age of approximately 1 year from a USDA-licensed animal dealer. They were trained to run on the treadmill. Each dog underwent two surgical implantations of 12–16 electromyographic (EMG) electrodes in the trunk muscles. Recording of muscle activity began on the second day after surgery and continued for 2–5 days. The electrodes were removed 5–8 days after implantation. The dogs recovered for at least 30 days before the second electrode implantation. After recovering from the removal of the second set of electrodes, the dogs were spayed and adopted

as pets. All procedures conformed to the guidelines of the Brown University Institutional Animal Care and Use Committee.

For surgery, subjects were initially anesthetized with an intravenous injection of sodium thiopental or propofol. They were then intubated with an endotracheal tube and maintained on a ventilator with oxygen and 1.5–2.0% isoflurane for the duration of the surgery. Incisions were made through the skin above the site of electrode placement and patch (Loeb and Gans, 1986) or sew-through electrodes were secured to the muscles of interest. Lead wires from the electrodes were passed subcutaneously to a dorsal exit point just caudal to the dorsal tips of the scapulae. Electromyographic signals were passed through shielded, lightweight cables (Cooner Wire Inc.), filtered above 1000 Hz and below 100 Hz, and amplified 5000 or 10 000 times with Grass P511 AC amplifiers. These data were stored in digital form at 2000 Hz on a PC or Macintosh computer.

Three sites in the intercostal musculature were monitored, two at the fourth intercostal segment and one at the ninth intercostal segment. Patch electrodes were placed between the interosseous external and internal intercostal muscles in the fourth and ninth intercostal segments. The fourth intercostal segment was chosen to represent intercostal muscles associated with ribs that have a direct attachment to the sternum (sternal ribs). The ninth intercostal segment was chosen to represent intercostal muscles attached to ribs that do not directly articulate with the sternum, but function as the attachment site for the diaphragm (diaphragmatic ribs). Electrodes were placed between the osseous portions of the ribs, at a mid-dorsal level. Specifically, the electrodes in the fourth segment muscles were positioned at the level of the insertion site of the serratus ventralis muscle. The electrodes in the ninth segment muscles were positioned at the same dorso-ventral level. The electrodes were constructed from 0.3 mm, multistranded, Teflon-insulated stainless-steel wire (Cooner Wire Inc.) sewn through 1 cm × 3 cm rectangles of 0.8 mm Silastic sheeting (Dow Corning). The exposed portions of the wires were separated by 1–2 mm and oriented at 90° to the fiber direction of the muscles.

Sew-through electrodes (Betts *et al.* 1976) were placed in the parasternal portion of the internal intercostal muscles between the cartilaginous portions of the sternal ribs at intercostal segment number 4 (the third electrode site). These electrodes were constructed from the same wire as the patch electrodes. An overhand knot was tied in one end of the two leads. A 1–2 mm length of the insulation was removed from one side of each wire and these uninsulated segments were staggered at approximately 6 and 8 mm distance from the knot. A small square (3 mm × 3 mm) of Silastic sheeting was threaded past the exposed portion of the wires to abut the knot. The two lead wires were sewn directly into the muscle, parallel to the muscle fibers, so that the exposed segments of the wires were inside the muscle and the Silastic button acted as a stop. A second Silastic button was then threaded to the point where the wires exited the muscle and secured in place with a square

knot in the leads. This provided a secure anchoring of the electrode, and the 1–2 mm staggering of the exposed segments parallel to the fiber orientation resulted in a dipole when the muscle was active.

Sample size varied for the different recording sites. The interosseous intercostal muscles at the fourth segment were sampled in all four dogs; two electrodes per muscle per dog. Recordings of the activity of the intercostals from the ninth intercostal segment were made in three dogs from a total of five electrodes for each muscle. Activity of the parasternal muscles from the fourth intercostal site was sampled in two dogs with two electrodes per dog.

Timing of inspiratory and expiratory airflow was monitored with a mask-mounted screen pneumotachograph. To allow the dogs to breathe and pant as naturally as possible, the mask covered the entire face and was big enough to allow the mouth to open and the tongue to hang out of the mouth. The mask was held in place by a snug collar around the neck and was

sealed around the head just in front of the ears. A bias flow was provided to maintain the quality of the respiratory gases in the mask. Pressure changes around the screen were measured using an Omega 160 differential pressure transducer, with a range of ± 507 Pa. These transducers have a time response that gives 90% of the signal in 1 ms. The transducer was mounted on the mask so that the silicon diaphragm was oriented in the sagittal plane. This isolated the diaphragm from the vertical and forward accelerations of the dog. Motion artifact due to acceleration of the transducer was found to be too small to measure when checked by running the dogs with the transducer disconnected from the pressure tube to the inside of the mask.

Locomotor events were video-recorded at 120 Hz using a PEAK high-speed camera. An analog signal of the locomotor cycle was obtained by monitoring the vertical acceleration of the trunk using an Omega 103 accelerometer mounted dorsally in the lumbar region. The video recordings were synchronized

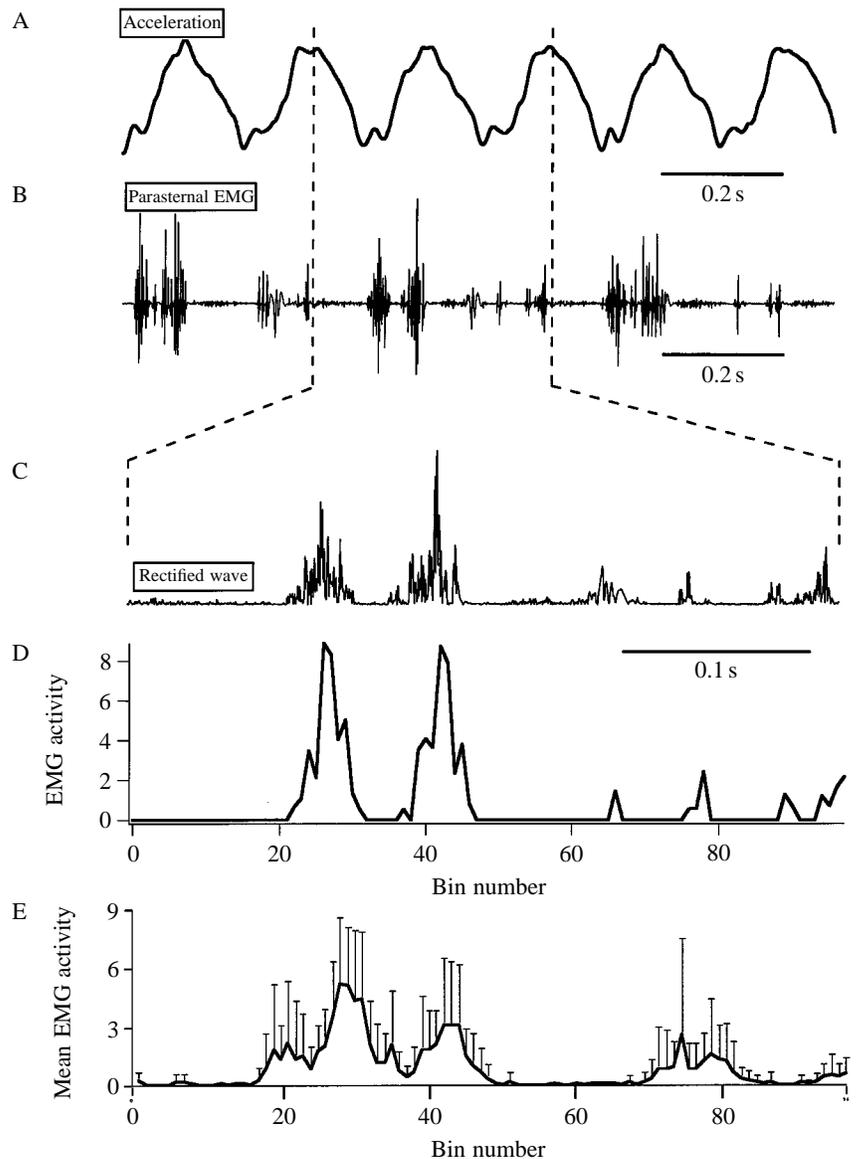


Fig. 1. Illustration of the method used to ensemble average the electromyographic signals. (A) The accelerometer (or pneumotachograph, not shown) signal was used to define the beginning and end of individual cycles. Each peak from the accelerometer trace represents one phase of limb support. Thus, two peaks represent a full locomotor cycle. (B) These times were used to define the electromyogram cycles. (C) Signals from the electromyogram cycles were rectified so that all spikes were positive. (D) The signal was divided into 100 bins and the EMG activity (Gans and Gorniak, 1980) was calculated for each bin. The EMG activity was calculated by multiplying the number of EMG spikes by the mean amplitude of those spikes. (E) The EMG activity was then averaged on a bin-by-bin basis for 17–22 consecutive ventilatory or locomotor cycles.

with the EMG, pneumotachograph and accelerometer recordings using a circuit that simultaneously illuminated a light-emitting diode in the view of the video camera and triggered data acquisition by the computer.

The relationship of muscle activity to the locomotor or ventilatory cycles was determined by ensemble averaging (Banzett *et al.* 1992a,b) of the electromyographic signals. The beginning and end times of individual cycles were determined from the pneumotachograph and accelerometer signals. These times were used to divide the electromyographic signals into locomotor and ventilatory cycle segments (Fig. 1). The electromyographic segments were then rectified so that all spikes were positive, partitioned into 100 bins, and the EMG activity was calculated for each bin by multiplying the number of spikes by the mean amplitude of the spikes (Gans and Gorniak, 1980). The EMG activity was then averaged on a bin-by-bin basis for 17–22 consecutive ventilatory or locomotor cycles. The resulting trace (see Fig. 1E) represents the mean EMG activity during an average locomotor or ventilatory cycle. If muscle activity was correlated with either locomotor or ventilatory function, distinct phasic bursts of EMG activity resulted when it was averaged relative to the cycle of the correlated function (e.g. Fig. 1E). If, however, muscle activity was not correlated with one of the two functions, averaging to

the cycle of that function produced a constant low-amplitude signal without phasic bursts.

Results

Intercostal activity during resting ventilation and panting

In all four dogs, activity of the intercostal muscles was correlated with ventilatory airflow when they were standing and breathing quietly or panting (Fig. 2). Activity in the parasternal intercostal muscles was always observed, but activity from the interosseous portions of the external and internal intercostal muscles was highly intermittent. In the interosseous intercostal muscles, the presence or absence of activity at a particular electrode site varied from trial to trial and even from minute to minute.

The phase relationship of the muscle activity relative to ventilatory airflow was largely the same during both resting ventilation and panting ventilation, with activity occurring slightly earlier in the ventilatory cycle during panting than during resting ventilation. Activity of the parasternal intercostal muscles was always associated with inspiratory airflow, beginning 30–50 ms before the start of inspiration. Activity from the external intercostal muscle from the fourth intercostal segment was also correlated with inspiration.

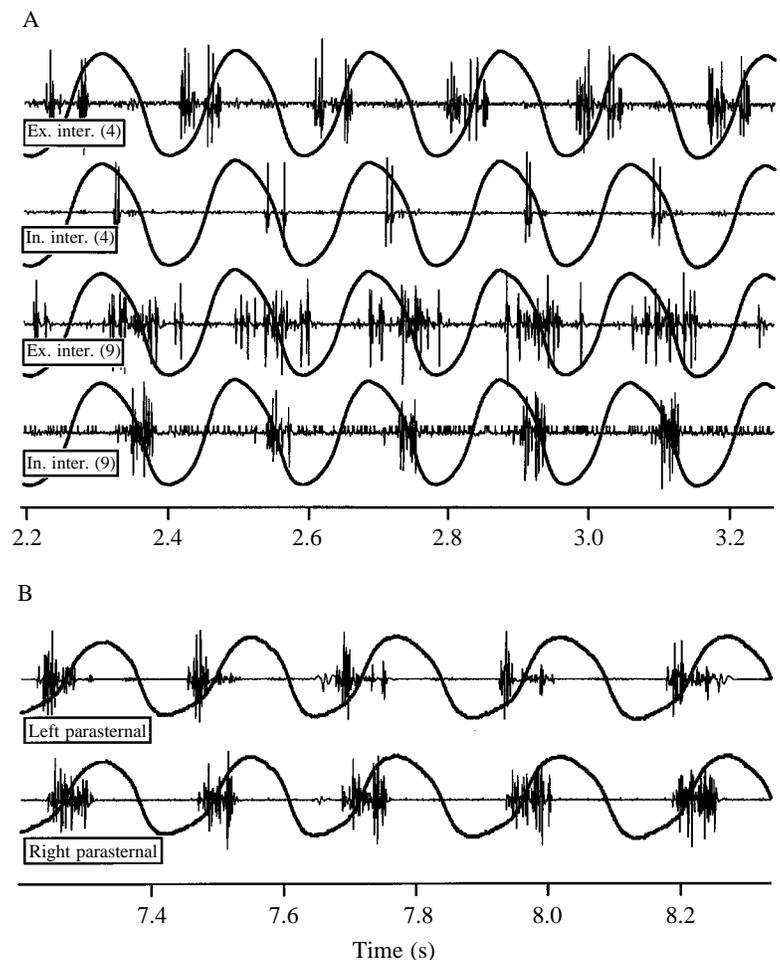


Fig. 2. Sample recordings of intercostal muscle activity during panting ventilation of standing dogs. In each case, the thick trace overlying the EMG trace is the signal from the pneumotachograph. Inspiration occurs when the trace is above the baseline of the EMG signal and expiration occurs when the trace is below the baseline. (A) Recordings from the interosseous portions of the intercostal muscles on the left side of the body. Recordings from the sternal intercostal muscles are from the fourth intercostal segment (4), and those from the diaphragmatic intercostal muscles are from the ninth intercostal segment (9). (B) Recordings from the parasternal intercostal muscles of a second dog from the fourth intercostal segment on the left and right sides of the body. For both dogs, breathing rate is approximately 5 Hz.

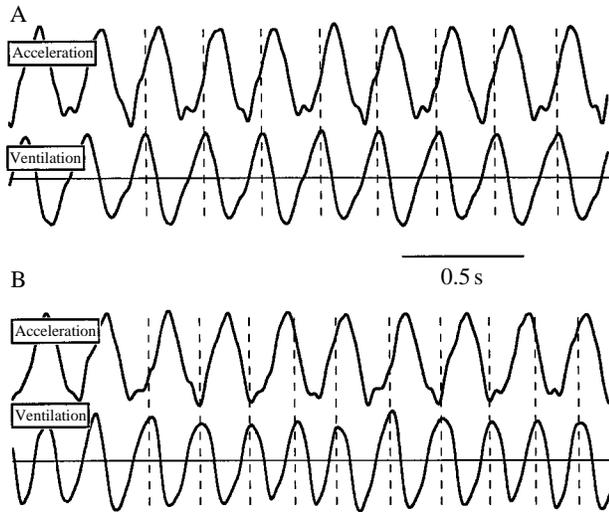


Fig. 3. Sample accelerometer (vertical acceleration) and pneumotachograph (ventilation) recordings during coupled (A) and uncoupled (B) breathing from a trotting dog. Compare the relative timing of the troughs in the accelerometer trace with the timing of the peaks in the pneumotachograph trace (dashed lines). Each peak from the accelerometer trace represents one phase of limb support. Thus, two peaks represent a full locomotor cycle. In both A and B, the dog is breathing at approximately 4 Hz. Inspiration occurs when the ventilation trace is above the baseline. In this dog, when ventilation is coupled to stride (A), peak inspiratory flow occurs prior to maximum vertical acceleration. In B, the ventilatory frequency is slightly higher than the step frequency, such that every fourth breath is correlated with the stride cycle in a manner similar to the pattern observed for each breath during coupled breathing. The horizontal time scale applies to both A and B.

However, activity from the external intercostal muscle from the ninth segment was associated with expiratory airflow in the three dogs from which this site was monitored. Activity from

the internal intercostal muscles from both the fourth and ninth segments was correlated with expiration.

Ventilation pattern during trotting

During trotting, the four dogs displayed a variety of breathing patterns. However, once they were warmed up and thermoregulating by 'panting' they breathed in a stereotypic pattern of two breaths per stride (Fig. 3). When breathing was coupled to the stride cycle, two patterns of airflow were observed. In two of the dogs, peak inspiratory flow occurred approximately 60–70 ms before peak vertical acceleration (Fig. 3A). When breathing in this way, inspiratory airflow occurred during the deceleration period and expiration occurred as the dogs accelerated upwards and forwards. In the other two dogs, inspiratory airflow occurred during the support phase of each step, with peak inspiratory airflow occurring 10–40 ms after the maximum vertical acceleration of the trunk (Fig. 4). In this case, peak expiratory flow occurred during the suspension phase of the step.

These two patterns of coupled breathing were the most common modes of breathing during trotting. However, brief episodes in which ventilation was not synchronized with the stride were also observed in all four dogs (Fig. 3B). These episodes of uncoupled breathing were transient in nature, lasting for as few as 5–10 ventilatory cycles or as long as 1–2 min. Generally, ventilatory frequency remained close to step frequency during uncoupled episodes. Although it is unclear why ventilation was at times uncoupled, uncoupled breathing did appear to correspond to periods of thermoregulatory transition. That is, when a dog trotting on the treadmill first started to pant, its breathing frequency was generally slower than its step frequency, such that breathing could not be coupled to stride. Similarly, the easiest way to elicit uncoupled breathing was to reduce the belt speed of the treadmill to a slow trot after a dog had warmed up and was panting vigorously. When this was done, breath frequency was

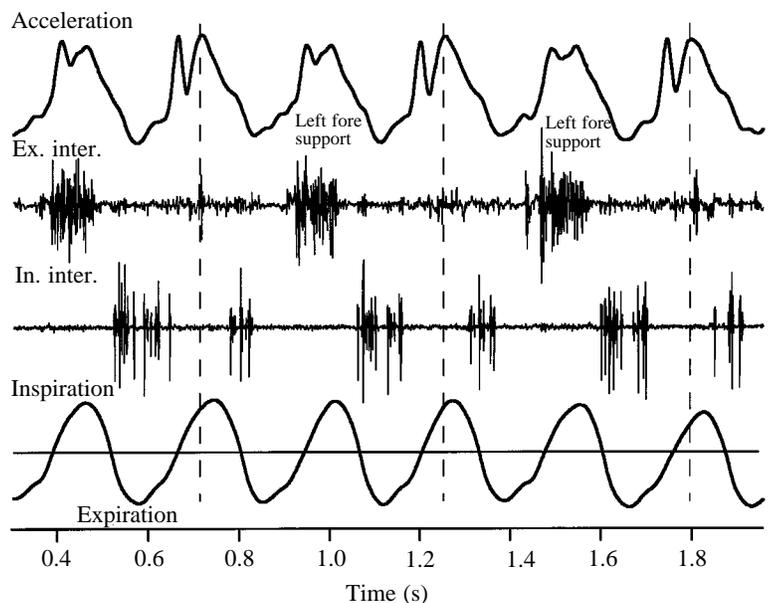


Fig. 4. Sample recording of muscle activity from left external and internal intercostal muscles from the fourth intercostal segment of a dog during trotting. The upper trace is the signal from the accelerometer. Each peak represents a phase of limb support. The second and third traces are EMG recordings from the external and internal intercostal muscles, respectively. The fourth trace is the signal from the pneumotachograph. Inspiratory airflow occurs when the trace is above the baseline. The dashed lines indicate different locomotor cycles.

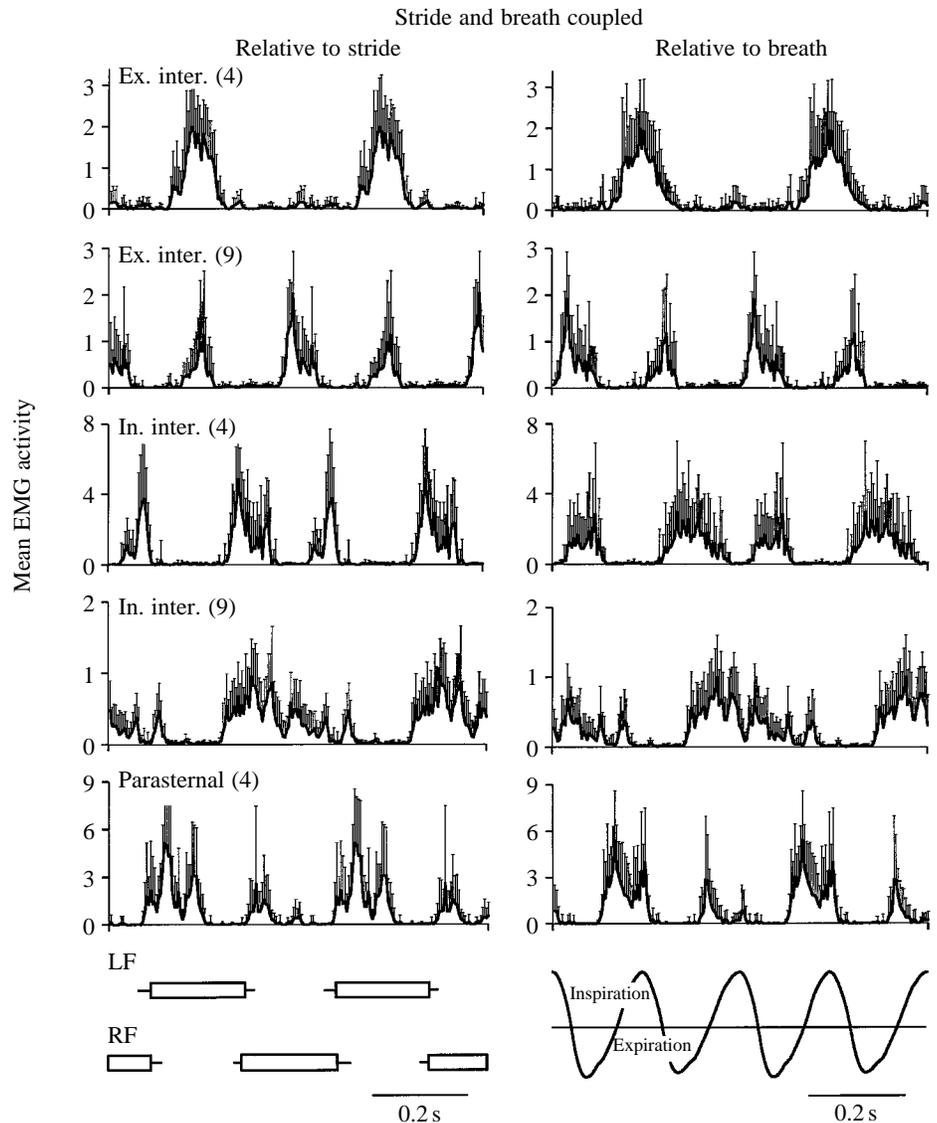


Fig. 5. Ensemble-averaged EMG signals from the left intercostal muscles of a trotting dog during coupled lung ventilation (see Materials and methods and Fig. 1). The signals are shown averaged relative to the stride cycle (left-hand column) or averaged relative to the breathing cycle (right-hand column). In each graph, the solid line represents the mean EMG activity from 17–22 locomotor or ventilatory cycles and the error bars represent one standard deviation. The boxes and error bars at the bottom of the left-hand column represent the mean duration and one standard deviation of forelimb support; LF, left forelimb, RF, right forelimb. The trace at the bottom of the right-hand column gives the timing of ventilatory airflow. Ex. inter., interosseous external intercostal muscle from the fourth (4) and ninth (9) intercostal segments. In. inter., interosseous internal intercostal muscle from the fourth (4) and ninth (9) intercostal segments. Parasternal, parasternal intercostal muscle from the fourth intercostal segment.

always higher than step frequency (e.g. Fig. 3B), with the result that the two cycles drifted in time relative to each other.

Intercostal activity during trotting

When ventilation was coupled to the locomotor cycle, activity of the intercostal muscles was phasically locked to the stride cycle in all four dogs (Figs 4, 5). In each muscle, there were one or two bursts of activity per locomotor cycle. This varied among the muscles and among the dogs. Generally, when there were two bursts of activity, one was of greater duration and amplitude than the other. The tight phase relationship between muscle activity and the locomotor events suggests that the function of these muscles is associated with the locomotor effort. However, when ventilation was coupled to the locomotor cycle, the timing of muscle activity was also tightly correlated with the timing of ventilatory airflow (Figs 4, 5). Furthermore, the timing of muscle activity relative to airflow was very similar to the timing during panting ventilation when the subjects were standing still. The external

intercostal muscles from the fourth intercostal segment and parasternal intercostal muscles were active during inspiration, whereas the external intercostal muscles from the ninth intercostal segment and the fourth internal intercostal muscles were active during expiration. Consequently, these results suggest that when ventilatory and stride cycles are coupled the intercostal muscles may contribute to the locomotor effort, may help produce ventilation or are simultaneously involved in both functions.

However, when breathing was not coupled to the locomotor cycle, the interosseous intercostal muscles remained phasically locked to the locomotor cycle and their activity was no longer correlated with ventilation (Fig. 6). This pattern was observed in all four dogs and in every example of uncoupled ventilation that was recorded. That is, no examples of a correlation between the activity of the interosseous intercostal muscles and the timing of ventilatory airflow were observed during uncoupled breathing. Ensemble averaging of the recorded signals illustrates this (Fig. 7). When the EMG activity was

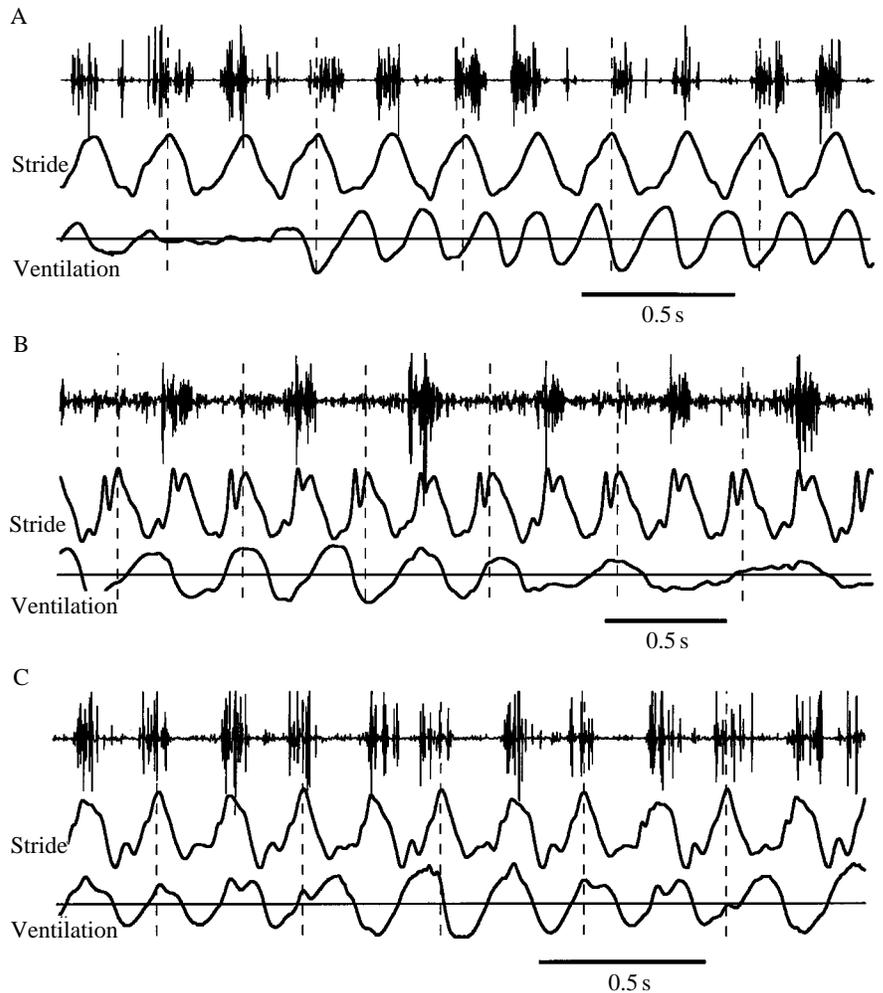


Fig. 6. Sample recordings from three dogs (A–C) of the activity of the external intercostal muscle from the fourth intercostal segment during periods when ventilation was not synchronized with the stride cycle. In each example, the upper trace is a recording of muscle activity, the middle trace is a recording of the vertical accelerations of the trunk and the third trace is a recording of ventilatory airflow. The dashed lines represent the same time in each locomotor cycle. In each dog, activity of the external intercostal muscle is correlated with the timing of peak vertical acceleration, but is not correlated with the timing of ventilatory airflow.

averaged relative to the locomotor cycle, distinct phasic bursts of activity were observed. This activity was essentially the same as that produced from trials in which ventilation and stride were coupled (see Fig. 5). In contrast, when the EMG activity was averaged relative to the ventilatory cycle, phasic bursts of activity were not found in the interosseous intercostal muscles, indicating that the activity of these muscles was not correlated with ventilation. The parasternal intercostal muscles exhibited the opposite pattern in the two dogs in which this muscle was monitored (Fig. 8). In every example in which ventilation was not coupled to the locomotor cycle, activity of the parasternal muscles remained correlated with ventilation. Ensemble averaging of parasternal signals from uncoupled trials showed that these muscles were phasically locked to inspiratory airflow and were not correlated with events of the locomotor cycle (Fig. 7). The hint of parasternal activity associated with the stride cycle in Fig. 7 is due to the fact that parts of the sequence that was averaged contained breaths that were timed appropriately relative to the stride cycle to appear coupled. The analysis was performed on the individual sequence for each muscle that showed the least coupling. For the case of the parasternal muscle, some coupling was present in the sequence. However, when the EMG activity averaged

relative to the stride cycle is compared with the muscle activity averaged relative to the breathing cycle, it is clear that the parasternal muscle stays coupled to ventilatory airflow when striding and breathing are uncoupled.

Discussion

Activity during panting and resting ventilation

During both resting and panting ventilation, activity of the intercostal muscles from the sternal site (fourth intercostal segment) was consistent with the traditional view of the functional role of these muscles in ventilation. That is, activity of the external intercostal muscles was associated with inspiration and activity of the internal intercostal muscles was timed appropriately to produce expiration. However, activity of these same muscles from the diaphragmatic site (ninth intercostal segment) displayed a different pattern. At the diaphragmatic site, activity of both external and internal intercostal muscles was associated with expiratory airflow. This, together with the observation from the lizard *Iguana iguana* that the two intercostal muscles produce inspiration but that neither produces expiration (Carrier, 1989), indicates that ventilatory function may not provide an adequate explanation

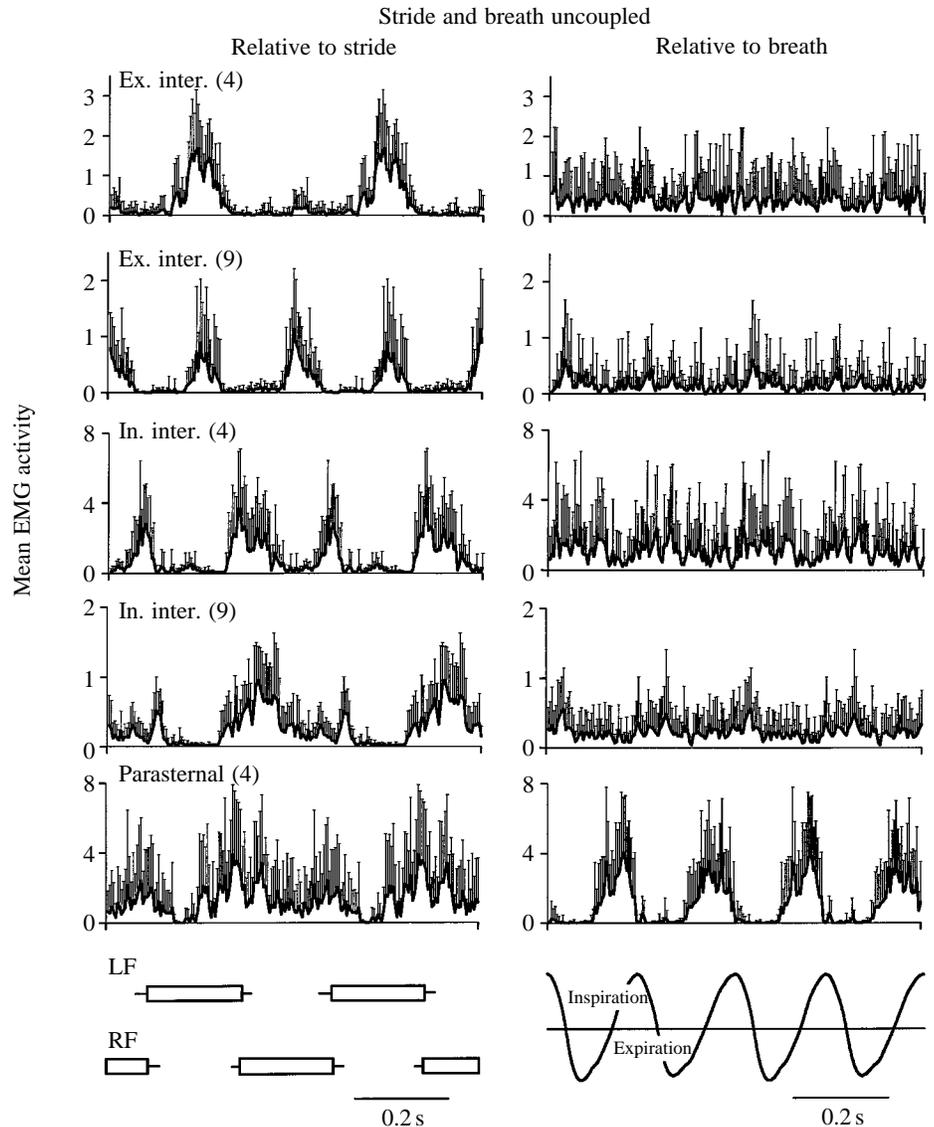


Fig. 7. Ensemble-averaged EMG signals from the left intercostal muscles of a trotting dog during uncoupled lung ventilation (see Materials and methods and Fig. 1). The signals are shown averaged relative to the stride cycle (left-hand column) or averaged relative to the breathing cycle (right-hand column). In each graph, the solid line represents the mean EMG activity from 17–22 locomotor or ventilatory cycles and the error bars represent one standard deviation of the activity. The boxes and error bars at the bottom of the left-hand column represent the mean duration and one standard deviation of forelimb support; LF, left forelimb, RF, right forelimb. The trace at the bottom of the right-hand column gives the timing of ventilatory airflow. Ex. inter., interosseous external intercostal muscle from the fourth (4) and ninth (9) intercostal segments. In. inter., interosseous internal intercostal muscle from the fourth (4) and ninth (9) intercostal segments. Parasternal, parasternal intercostal muscle from the fourth intercostal segment.

for the distinct orientations of the intercostal muscles, or even for the presence of two separate intercostal muscle layers (De Troyer *et al.* 1985).

Function of the intercostal muscles during trotting

The typical pattern of breathing in a trotting dog is one breath per step, which is two breaths per locomotor cycle (Bramble and Jenkins, 1994; this study). Because breathing is coupled to the stride cycle, activity of the intercostal muscles is correlated with both the locomotor and ventilatory events. Activity of the external intercostal muscles from the sternal ribs coincides with the beginning of inspiratory airflow, and activity of the diaphragmatic external intercostal and sternal internal intercostal muscles is associated with expiratory flow. Thus, it would be reasonable to assume that the activity of these muscles during trotting contributes to the production of ventilatory airflow. However, when breathing is not coupled to locomotion, activity of the interosseous intercostal muscles remains correlated with the stride cycle and, consequently, it

phase-shifts relative to the ventilatory events. Under these circumstances, the external and internal intercostal muscles appear to abandon any contribution they might make to ventilation. This indicates that, in trotting dogs, locomotion is the predominant function of the interosseous external and internal intercostal muscles. The role that these muscles are likely to play in locomotion is stabilization of the rib cage and trunk so that the appendicular muscles have a solid base from which to apply forces to the limbs and ground.

In contrast, activity of the parasternal muscles is correlated with inspiratory airflow when breathing and stride are not coupled. The parasternal muscles are the most ventral portion of the internal intercostal muscles and are recognized as acting to produce inspiration in resting mammals (Taylor, 1960; De Troyer *et al.* 1988; De Troyer and Farkas, 1989; De Troyer, 1991). Their entrainment to the ventilatory cycle during episodes of uncoupled breathing indicates that, in trotting dogs, their dominant function is ventilation. A solely ventilatory function for these muscles is not shared by lizards. In green

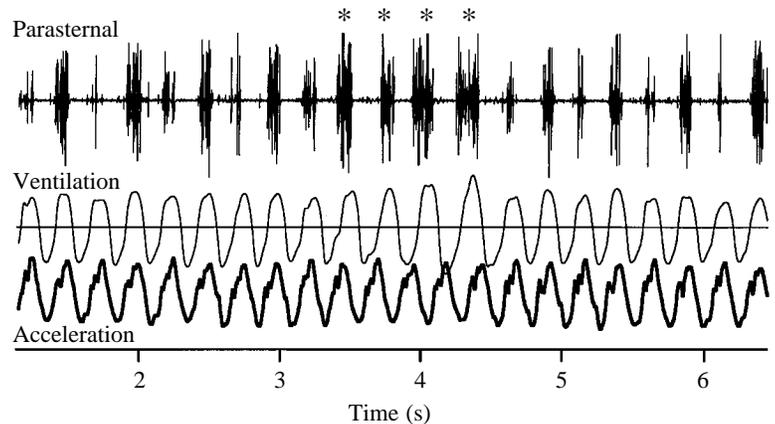


Fig. 8. Sample recording of the fourth parasternal intercostal muscle in which ventilation becomes uncoupled from the stride cycle midway through the trial. Activity of the parasternal muscle remains correlated with the timing of inspiratory airflow, even during the four breaths in the middle of the trace (asterisks) in which ventilation and stride are not coupled.

iguanas, *Iguana iguana*, the homologous portions of the internal intercostal muscles cease inspiratory function and adopt a locomotor role at the initiation of locomotor movements (Carrier, 1990, 1991).

The observation in trotting dogs that the interosseous intercostal muscles are predominantly locomotor muscles is unexpected, given the traditional view of their ventilatory function. However, this observation is less surprising from a historical perspective. As mentioned above, the obliquely oriented hypaxial muscles of fishes are not used for lung ventilation. In fishes, these muscles probably contribute to lateral bending of the trunk and provide torsional stability during swimming. In salamanders, the obliquely oriented hypaxial muscles (i.e. the external oblique, internal oblique and transversalis muscles) are active during terrestrial walking in a manner that indicates that they stabilize the trunk against long-axis torsion induced by gravity acting through diagonal limb support (Carrier, 1993). In *Iguana iguana*, the intercostal muscles and oblique muscles display a pattern of activity during walking that is essentially the same as that observed in the oblique hypaxial muscles of walking salamanders (Carrier, 1990). Here, also, the suggestion is that the intercostal and oblique muscles provide torsional stability during walking. However, there is recent evidence indicating that the external oblique muscles of lizards do not contribute to torsional stability during locomotion, but play a role in lateral bending of the trunk (Ritter, 1995). The ancestral function of the hypaxial muscles was locomotion and, although these muscles subsequently became important agonists of ventilation, observations in salamanders, lizards and dogs indicate that locomotion remains their primary function.

Implications for the evolution of locomotor stamina

Early in the evolution of terrestrial vertebrates, a conflict arose between locomotion and ventilation. As argued above, the ancestral function of the hypaxial muscles was locomotion. However, in early tetrapods, the hypaxial muscles also became solely responsible for the function of lung ventilation. Whether the first amphibians were aspiration breathers (Gans, 1970) or retained the buccal pumping mechanism of fishes (Brainerd *et*

al. 1993), we can be confident that ventilation of the lungs by the hypaxial musculature arose very early in the history of tetrapods, because all modern amniotes are aspiration breathers. When the shift from buccal pumping to aspiration breathing occurred, the hypaxial muscles became responsible for two separate and apparently conflicting functions. In some lizards, such as *Iguana iguana*, these competing demands result in reduced lung ventilation and gas exchange during locomotion at speeds exceeding the very low speeds that they can sustain (Carrier, 1987a, 1991; Wang *et al.* 1995). On the basis of the skeletal anatomy and mode of locomotion of early tetrapods, combined with what we know about the function of the axial muscles in extant tetrapods, it can be argued that the early tetrapods were also unable to run and breathe at the same time (Carrier, 1987b, 1991). If early tetrapods suffered this constraint, what changes evolved in the lineages that gave rise to birds and mammals that made simultaneous running and breathing possible?

One feature of birds and mammals that may facilitate simultaneous locomotion and breathing is a mechanical and neurological coupling of the two functions. Although birds and mammals have been observed to breathe in an uncoupled pattern while running, there is increasing evidence that maintenance of a constant phase relationship between locomotor and ventilatory events is the normal pattern. The pattern most often used by trotting dogs (Bramble and Jenkins, 1994; this study) and running fowl (Nassar, 1994) appears to be one breath per step. In both species, inspiration usually occurs during the first half of limb support as the animal decelerates, and expiration occurs during the second half of the support phase and during the swing phase. There is overwhelming evidence for a strict one breath per stride coupling pattern in placental mammals when they gallop (Hornicke *et al.* 1983; Attenburrow, 1982; Bramble and Carrier, 1983; Young *et al.* 1992) and in macropod marsupials when they hop bipedally (Baudinette *et al.* 1987). Bats also exhibit a consistent ratio of one breath per locomotor cycle when they fly (Suther *et al.* 1972; Thomas, 1981). Flying birds (Berger *et al.* 1970; Butler, 1982; Boggs *et al.* 1993) and bipedally running humans (Bramble and Carrier, 1983; Bramble, 1983) display a greater variety of breathing patterns,

but a constant phase relationship is usually maintained between the timing of locomotor events and ventilatory airflow. Thus, in those tetrapods that are capable of sustaining vigorous locomotion, there tends to be a coordinated phase-locking of the locomotor and ventilatory cycles.

Currently, there is controversy over the functional significance of locomotor-ventilatory coupling. There are questions about the extent to which the locomotor movements produce ventilatory airflow (Banzett *et al.* 1992*a,b*), as well as concerns that locomotor-respiratory coupling may simply be a consequence of two neural oscillators becoming entrained (Banzett *et al.* 1992*a*). However, the observations that the intercostal muscles of lizards and the interosseous intercostal muscles of dogs have a locomotor role that is predominant over their ventilatory function suggests that locomotor-ventilatory coupling does serve an important function. The results of the present study indicate that, when ventilation is not coupled to stride in trotting dogs, the interosseous intercostal muscles do not participate in the production of lung ventilation and the parasternal intercostal muscles do not participate in locomotion. For these muscles, and possibly other hypaxial muscles, simultaneous participation in both locomotion and ventilation may require an integrated coupling of the two functions.

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