

EFFECTS OF CHANGES IN LOCOMOTOR INTENSITY, HYPOXIA AND HYPERCAPNIA ON LOCOMOTOR- RESPIRATORY SYNCHRONY DURING WALKING/RUNNING IN CANADA GEESE

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Summary

To examine the effects of locomotor frequency and respiratory drive on the incidence of locomotor-respiratory coupling (i.e. entrainment), we examined the relationship between locomotor and ventilatory patterns in trained Canada geese that were: (1) running at three speeds (0.40, 0.52 and 0.72 m s⁻¹) on a treadmill, (2) free-running at three velocity intervals (0.40–0.72, 1.0–1.5 and 1.5–2.0 m s⁻¹) overground, and (3) breathing hypoxic (12% O₂/1% CO₂) and hypercapnic (5% CO₂/30% O₂) gas mixtures while running at one speed (0.52 m s⁻¹) on the treadmill.

The portion of the time that the locomotor and respiratory systems were coupled was significantly greater during overground locomotion (41.1 ± 3.2%) than at comparable speeds on the treadmill (29.0 ± 2.6%). In addition, a significant increase in coupling with velocity was only observed during overground locomotion (41.1 ± 3.2% at 0.40–0.72 m s⁻¹ vs 57.7 ± 6.3% at 1.5–2.0 m s⁻¹). This increase in entrainment appeared to be the direct result of an increase in locomotor frequency *per se* and not due to the increase in metabolic rate associated with greater locomotion velocity.

The effects of hypercapnia on the degree of entrainment were unclear owing to inter-animal variability; however, hypoxia caused a substantial decrease in entrainment from air-breathing values (34.8 ± 3.9%) to levels not different from those possible due to chance alone (20%).

Thus, it appears that locomotor pattern does entrain ventilatory rhythm and that the degree of entrainment is partially dependent on limb movement frequency. However, the increased respiratory drive associated with hypoxia appears to override the influence of locomotor rhythm on respiratory pattern, with a subsequent decrease in entrainment.

Introduction

Examples of locomotor-respiratory coupling (entrainment) abound in the

words: locomotor-respiratory coupling, entrainment, hypoxia, hypercapnia, exercise hyperpnea, goose.

literature. There is now evidence of entrainment in fish (Webb, 1975), birds (Hart & Roy, 1966; Funk *et al.* 1989) and at least six groups of mammals (primates, Carnivora, Perissodactyla, Lagomorpha; Bramble & Carrier, 1983; Marsupialia; Baudinette *et al.* 1987; and Chiroptera; Suthers *et al.* 1972). However, very little is known of the mechanisms controlling entrainment.

In a general sense, entrainment can be described as the coupling of two non-linear oscillatory systems. The interactions between the two systems time-lock the activity patterns of each system at the same frequency or some harmonic thereof (Yonge & Petersen, 1983). The interactions between the two oscillators could be reciprocal, each oscillator affecting the output of the other, or unidirectional, a driving oscillator forcing the second oscillator to the frequency of the first (Viala, 1986). Although there is some evidence that reciprocal interactions between respiratory and locomotor pattern generators are involved in the coupling of locomotor and respiratory rhythms (Viala, 1986), it is generally accepted that locomotor rhythm influences respiratory rhythm (Iscoe & Polosa, 1976; Bechbache & Duffin, 1977; Yonge & Petersen, 1983; Paterson *et al.* 1986). The influence of locomotion on respiration may occur through efferent connections at spinal (Viala *et al.* 1979; Viala, 1986) or supraspinal levels (Eldridge *et al.* 1985; Viala *et al.* 1987) or *via* the influence of limb afferent feedback on central respiratory networks (Kalia *et al.* 1972; Iscoe & Polosa, 1976; Cross *et al.* 1982; Waldrop *et al.* 1986).

Experiments examining the effects of limb proprioception on entrainment in humans during cycling and running activity have yielded inconsistent results. Some indicate an effect of limb movement frequency on entrainment (Bechbache & Duffin, 1977), others indicate an effect of metabolic work rate (Jasinkas *et al.* 1980), while still others show neither (Kohl *et al.* 1981; Paterson *et al.* 1986). At the centre of this problem has been the failure to examine the effects of increased limb movement rate on entrainment rate while maintaining a constant metabolic work rate, and *vice versa* (Bramble & Carrier, 1983). Furthermore, in the steady state of moderate exercise, ventilation has been shown to be tightly coupled to metabolic CO₂ production (see review by Wasserman *et al.* 1986). Thus, when limb movement rate and metabolic rate are allowed to change concomitantly, it is not possible to distinguish between locomotor drive, metabolic rate and ventilatory drive as the factor affecting the degree of locomotor-respiratory coupling. Recent experiments with geese, however, have shown that when stride frequency is constant, entrainment rates are independent of the changes in oxygen uptake (\dot{V}_{O_2}), CO₂ output (\dot{V}_{CO_2}) and ventilation produced through increased work rate (Funk *et al.* 1989). As a consequence, geese offer an opportunity to examine the independent effects of locomotor drive on entrainment.

Other non-exercise drives to ventilation, such as hypoxia (Paterson *et al.* 1987) have also been shown to affect the coupling of locomotor and respiratory systems. Thus the goals of this study were: (1) to determine whether increasing locomotor movements enhance entrainment and (2) to examine the effects of increased chemical drives to respiration on entrainment in Canada geese. To this end we

examined the relationship between locomotor and respiratory patterns: (1) while birds walked/ran over a range of velocities on a treadmill and overground and (2) while the birds respired normal air, and hypoxic and hypercapnic gas mixtures during treadmill walking.

Materials and methods

Treadmill experiments

Training and equipment

The training procedures and equipment used to measure minute ventilation (\dot{V}_E), tidal volume (V_T), breathing frequency (f_v), oxygen uptake (\dot{V}_{O_2}), CO_2 output (\dot{V}_{CO_2}), mixed expired gas concentrations (PE_{O_2} and PE_{CO_2}) and stride frequency (f_s) during treadmill running have been described, in detail, previously (Funk *et al.* 1989).

Briefly, four Canada geese (*Branta canadensis*; 4.2 ± 0.2 kg) were raised from hatching. The goslings spent at least 1 h per day wearing a facemask and were run on the treadmill at least once per week until they were 3 months old. Two additional months of daily treadmill training was then started. The birds were fitted with a form-fitting mask (60 g) to which a pneumotachograph (Fleisch model no. 00) and associated gas delivery lines could be easily attached. The mask and pneumotachograph had a combined dead space volume of 4.0 ml, and therefore increased total dead space by approximately 10% (Fedde *et al.* 1986).

The pneumotachograph was attached to a differential pressure transducer (Validyne DP 103-18) and an integrating amplifier (Gould) in order to obtain tidal volume (V_T), breathing frequency (f_v) and minute ventilation (\dot{V}_E). The pneumotachograph was heated to prevent condensation and calibrated by injection and withdrawal of known gas volumes through the mask before and after each run.

Expired gas fractions were monitored using a paramagnetic O_2 analyzer (Beckman OM-11) and infrared CO_2 analyzer (Beckman LB- CO_2), connected in series, with sampling from the distal end of the pneumotachograph at 600 ml min^{-1} . Oxygen uptake (\dot{V}_{O_2}) and CO_2 output (\dot{V}_{CO_2}) were measured by passing 10 l of air past the end of the pneumotachograph through lightweight anesthetic tubes and collecting the effluent in a 2-l Douglas bag. The fractional concentrations of O_2 and CO_2 in the mixed gas were determined using the O_2 and CO_2 analyzers described above. \dot{V}_{O_2} and \dot{V}_{CO_2} were then calculated using the Fick equation. Hypoxic and hypercapnic gas mixtures were controlled using precision gas flow meters, tested using the O_2 and CO_2 analyzers described above, and delivered at 10 l min^{-1} via the system previously described for the measurement of \dot{V}_{O_2} and \dot{V}_{CO_2} .

To reduce visual disturbance, the birds were run in a plywood box overlying a variable-speed, motor-driven treadmill. Stride frequency was recorded from an amplified signal originating from a force plate under the moving treadmill belt.

Protocol

The birds were run on the treadmill at 0.40, 0.52 and 0.72 m s^{-1} . \dot{V}_E , V_T , f_V , \dot{V}_{O_2} , \dot{V}_{CO_2} , P_{EO_2} , P_{ECO_2} and f_s were measured continuously for 1 min pre-activity, 8 min of walking and 5 min of recovery. Values of \dot{V}_E , V_T , f_V and f_s represent means (\pm s.e.) of seven runs on four animals and \dot{V}_{O_2} and \dot{V}_{CO_2} values represent means (\pm s.e.) of three runs on three animals.

In another series of experiments, the birds were run at 0.52 m s^{-1} while breathing one of three different gas mixtures: (1) air, (2) 5% CO_2 and (3) 12% O_2 . The 5% CO_2 was administered on a hyperoxic background of 30% O_2 /balance N_2 . Brackenbury (1986) exposed running ducks to 10% O_2 and found a 0.53 kPa (4 mmHg) decrease in P_{aCO_2} . Addition of 3% CO_2 to the mixture during exercise resulted in a 0.13 kPa (1 mmHg) increase in P_{aCO_2} relative to resting levels. Thus, in this study, the 12% O_2 mixture was delivered in a background of 1% CO_2 to minimize the large decrease in P_{aCO_2} associated with hypoxic exercise without causing an acidosis. Using the same apparatus described above, preliminary gas response studies were performed on resting animals ($N = 4$), sitting quietly in a small holding pen, for later comparison with the gas responses observed during exercise. These studies indicated that the level of \dot{V}_E attained following 2 min of exposure to a new gas was not different from that seen after 10 min of exposure. Jones & Purves (1970) also found that 90% of the increase in \dot{V}_E associated with inspiration of 4% CO_2 occurs within the first 48 s of exposure. Thus, following 2 min of exposure to a new gas mixture, \dot{V}_E , V_T , f_V , \dot{V}_{O_2} and \dot{V}_{CO_2} were monitored for 1 min pre-activity, 8 min of walking and 5 min of recovery during the administration of the 5% CO_2 and 12% O_2 gas mixtures. All values represent means (\pm s.e.) of three runs on three animals.

Overground, free-running experiments

Training and equipment

To examine the differences in locomotor-respiratory coupling between treadmill running and overground free running, as well as examine the effects of f_s on entrainment over a larger velocity range than was possible on the treadmill, a second group of geese was raised from hatching. This group was treated virtually the same as the previous group. For their first 3 months they wore a cylindrical face mask for 1 h per day. These birds were also fitted with lightweight nylon harnesses. A canvas strap was attached to the mesh harness and passed just caudal to the wings onto the middle of the back. The harness did not interfere with wing or leg movements in any way.

At 3 months of age, the birds were introduced to a walled circular walkway (Fig. 1). In the centre of the circular walkway was a double aluminum pole. The outer pole was fixed while the center pole rotated within the outer pole. The center pole supported a cross-beam which had a counterweight at one end and an L-shaped bar at the other. This additional bar (L-bar) supported a decoy goose at the leading end. The geese were tethered to the trailing end of the L-bar via a 200 cm

tether that clipped to the canvas strap on the harness. The chain contained two sets of recording leads. One set carried breathing frequency information from a thermistor (Fenwall 112-202EAJ-B01) placed in the mask, overlying a nostril, to a

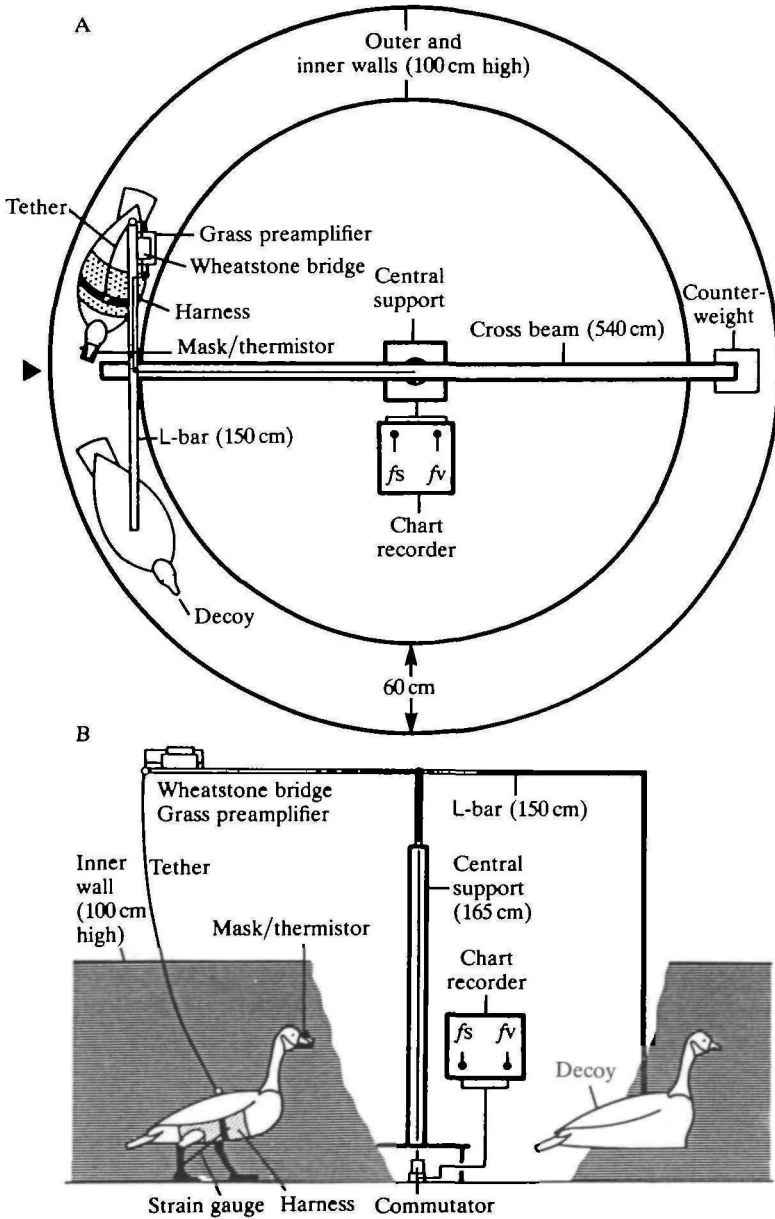


Fig. 1. Schematic diagram of the apparatus used in the free-running experiments as seen in top view (A). The arrowhead in A (▶) indicates the angle from which the side view (B) was taken. In the side view, the outer wall is not shown and the inner wall has been cut away to show the central support. f_s , stride frequency; f_v , breathing frequency.

Wheatstone bridge that was attached to the trailing end of the L-bar. The mask had a dead space volume of less than 2.0 ml, and thus increased total dead space by less than 5%. The second set of leads carried the signal from a strain gauge to a preamplifier (Grass model P15D) and indicated stride frequency. The birds were not bothered by the chain and leads and eagerly chased the decoy goose around the circular walkway.

A major objective of this part of the study was to ensure that we did not force (i.e. pace) the locomotor rhythm, as occurs on a moving treadmill. Thus the 'walker' device was not motor driven. The experimenters manually rotated the system within a given velocity range, but each bird ultimately selected its own precise velocity and locomotor rhythm. Forward tension was never placed on the chain. An event marker indicated the completion of each lap and allowed calculation of average locomotor velocity per revolution.

Protocol

Stride frequency and f_v were recorded during running at three velocities. Since we did not want to force locomotor rhythm and the birds did not always run at a constant velocity, the three speed levels encompassed fairly wide ranges. The first range encompassed the speeds produced on the treadmill (0.40–0.72 m s⁻¹). The second range varied between 1.0 and 1.5 m s⁻¹, while the third range varied from 1.5 to 2.0 m s⁻¹. The birds were run for a minimum of 8 min at the lowest speed, 5 min over the middle range, and 4 min at the highest velocity. One replicate was performed, so that values of f_s , f_v , and percent entrainment obtained during free running represent means (\pm s.e.) of 10 trials on five animals.

Data analysis

The degree of locomotor–respiratory entrainment was determined as described by Funk *et al.* (1989). Stride frequency (f_s) and breathing frequency (f_v) were measured for each 10-s interval throughout the periods of treadmill walking/running and overground walking/running. As entrainment is defined as a close integer relationship between f_s and f_v (Paterson *et al.* 1987), coupling ratios (f_s/f_v) were calculated for each 10-s interval to determine the degree of entrainment. Assuming random association of f_s and f_v and assigning confidence limits of ± 0.05 for entrained f_s/f_v values, chance coupling of locomotor and breathing patterns could reach 20% (Paterson *et al.* 1987). Percent entrainment data were normalized using the arcsin transformation before a *t*-test was used to ascertain whether the incidence of entrainment was statistically greater than the maximum possible by chance (20%) (Paterson *et al.* 1987).

Respiratory data collected from the treadmill experiments were averaged over the last 4 min of each run prior to comparison. Values of f_s , f_v and percent entrainment from the free-running experiments represent average values recorded over the course of each trial. Since the lowest velocity range observed during overground running encompassed the entire range of speeds produced on treadmill, all f_s , f_v and entrainment data from the treadmill experiments were

pooled (i.e. 21 measurements from four animals) prior to comparison with the free-running values of f_s , f_v and percent entrainment. Unless otherwise stated, ANOVA and Tukey's multiple comparison test were used to test the differences between means of all entrainment and respiratory data. Values of $P < 0.05$ were assumed to be significant.

Results

Effects of treadmill speed on entrainment

\dot{V}_E increased approximately 2.5–3.0 times immediately upon the onset of treadmill walking (Fig. 2). V_T remained unchanged from resting levels and thus the change in f_v was solely responsible for the initial increase in \dot{V}_E from rest to walking. Increases similar in magnitude to those seen in \dot{V}_E were also observed in \dot{V}_{O_2} and \dot{V}_{CO_2} with the onset of treadmill walking. The magnitude of these initial increases in \dot{V}_E , f_v , \dot{V}_{O_2} and \dot{V}_{CO_2} appeared to be independent of treadmill velocity.

Conversely, 'steady-state' levels of these variables (i.e. values taken over the last 4 min of the trial) were affected by treadmill velocity. The increase in velocity from 0.40 to 0.72 m s^{-1} produced small, further increases in \dot{V}_{CO_2} and \dot{V}_{O_2} and significant increases in \dot{V}_E , f_v and f_s (Figs 2, 3). Again f_v was solely responsible for the increase in \dot{V}_E associated with the increases in velocity. Breathing frequency continued to rise throughout the last 4 min of treadmill walking at all speeds, the degree depending on velocity ($20.5 \pm 12.6\%$ at 0.40 m s^{-1} ; $26.9 \pm 10.8\%$ at 0.52 m s^{-1} ; $30.1 \pm 7.1\%$ at 0.72 m s^{-1}). V_T fell marginally over the last 4 min of each run, at all treadmill speeds, such that \dot{V}_E remained relatively constant at 0.40 and 0.52 m s^{-1} (Fig. 2). At 0.72 m s^{-1} however, \dot{V}_E increased continuously during the last 4 min as f_v continued to increase more than V_T decreased (Fig. 2).

Locomotor and respiratory patterns displayed significant coupling at all velocities. All entrainment was harmonic, and coupling ratios of 1.5 and 2 strides breath^{-1} were the most common; however, coupling ratio values ranged from 2:1 to 6:1, the higher ratios occurring at the onset of treadmill locomotion. Entrained periods varied in duration from 10 s (experimental minimum) to 2 min, but were most frequently in the 30–60 s range. Several such periods were seen during each trial. The distribution of entrained periods did not appear to be biased towards any particular portion of the trials. Entrainment was as likely to occur at the onset of a trial as at the end. There were no statistically significant effects of treadmill velocity on the percentage of entrainment or the coupling ratio. However, a slight increase in coupling from 28.3 to 34.8% accompanied the increase in treadmill velocity from 0.40 to 0.52 m s^{-1} . As velocity was increased further to 0.72 m s^{-1} , however, entrainment returned to 27.1% (Fig. 4, bottom panel).

Effects of treadmill and overground locomotion on entrainment

Stride frequency increased similarly during both treadmill walking and overground walking (Fig. 4). However, the f_v recorded with the form-fitting mask and

pneumotachograph on the treadmill were significantly greater than those measured with the mask and thermistor used during the overground series of experiments under conditions of both rest and exercise. This difference may have been related to the slightly greater dead space or resistance of the form-fitting mask, although increases in dead space (Fedde *et al.* 1986) and resistance (Hof *et al.* 1986) have not been associated with increases in f_v . Alternatively, the form-

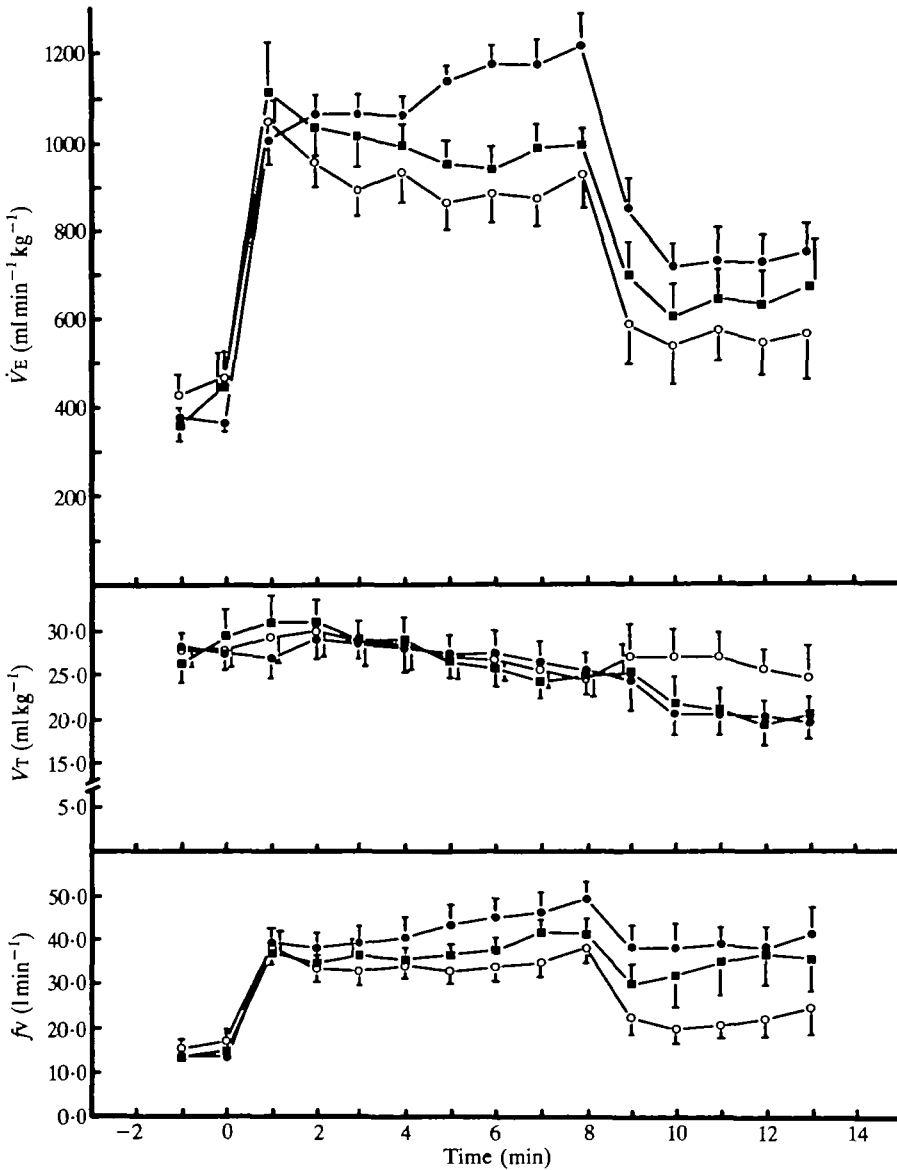


Fig. 2. Effect of treadmill walking/running at 0.40 m s^{-1} (○), 0.52 m s^{-1} (■) and 0.72 m s^{-1} (●) on minute ventilation (\dot{V}_E), tidal volume (\dot{V}_T) and breathing frequency (f_v) in Canada geese (four birds, $N = 7$; mean \pm s.e.).

fitting mask may have reduced the potential for evaporative heat loss which, depending on ambient temperature, can play a significant role in thermoregulation (Hudson & Bernstein, 1981). Aside from the shift in baseline f_v , however, it can be seen from Fig. 4 that f_v rises similarly with velocity during both treadmill and overground running, regardless of the method used to measure it.

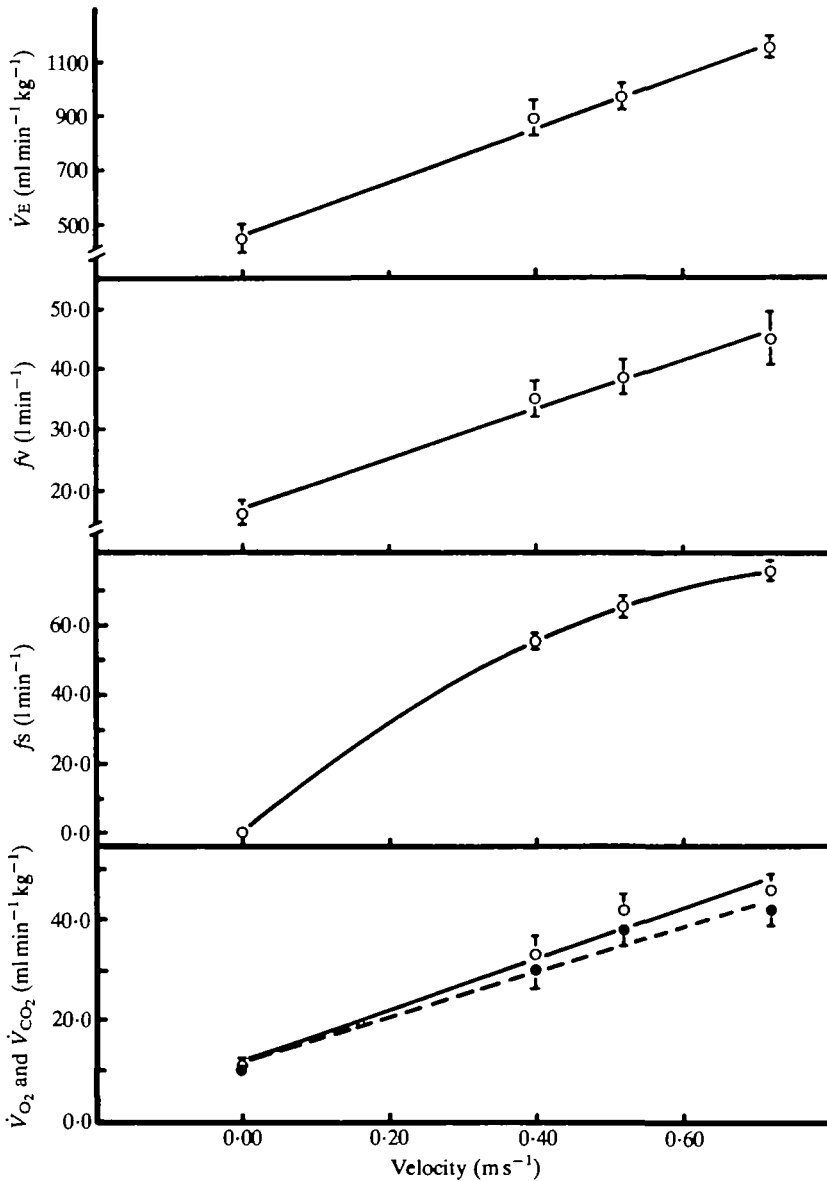


Fig. 3. Effect of treadmill velocity on mean levels of minute ventilation (\dot{V}_E), breathing frequency (f_v), stride frequency (f_s) (four birds, $N = 7$), O_2 uptake (\dot{V}_{O_2} , \circ) and CO_2 output (\dot{V}_{CO_2} , \bullet) (three birds, $N = 3$) averaged over the last 4 min of 8-min walking/running trials (mean \pm s.e.).

As seen during treadmill running, locomotor and respiratory rhythms displayed significant coupling at all velocities during overground locomotion. All entrainment was harmonic and, as seen at speeds similar to those observed during

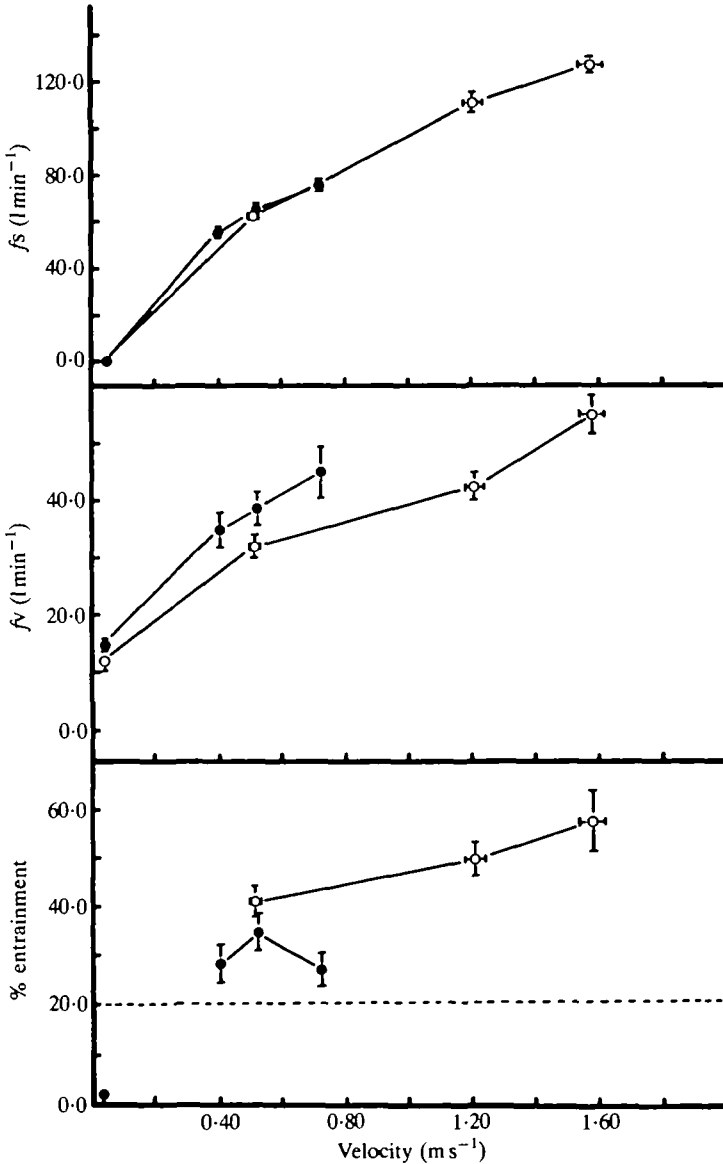


Fig. 4. Effect of locomotor velocity during free running (○) (five birds, $N = 10$) and treadmill running (●) (four birds, $N = 7$) on stride frequency (f_s), breathing frequency (f_v) and the degree of locomotor-respiratory coupling (% entrainment; dashed line shows 20%, the maximum entrainment predicted by chance) (data points = mean \pm s.e.). Since the lowest velocity range employed during free-running encompassed all three speeds used during treadmill running, all data from the treadmill experiments were pooled prior to comparison with free-running values.

treadmill running, the coupling ratio of 2 strides breath⁻¹ was most common. In the middle velocity range (1.0–1.5 m s⁻¹), the most common ratio was 2.5 strides breath⁻¹, while at the highest speeds (>1.5 m s⁻¹), 3.0 strides breath⁻¹ were most commonly observed. Coupling ratio values ranged from 1.5:1 to 4:1 (i.e. 3 steps breath⁻¹ to 8 steps breath⁻¹). Entrained periods varied in duration from 10 s to complete entrainment from start to finish (4 min in total, seen in one animal on one occasion).

The degree of coupling between locomotor and respiratory rhythms was significantly greater during overground running than during treadmill running. In addition, there was a significant increase in the degree of entrainment when running velocity was increased from 0.51 to 1.58 m s⁻¹ during overground locomotion (one-tailed *t*-test, $P < 0.026$).

Effects of inspired gas composition on entrainment

Fig. 5 illustrates the steady-state ventilatory responses of the birds to hypoxia and hypercapnia under rest and exercise conditions. Since the resting values of \dot{V}_E , V_T and f_v shown in Fig. 5 were taken from a separate series of experiments (see Materials and methods), they do not correspond exactly to the values seen at rest in Fig. 2. Under resting conditions, exposure to 5% CO₂/30% O₂ resulted in a 20% increase in f_v and a significant 80% increase in V_T , resulting in a significant 100% rise in \dot{V}_E (Fig. 5). During treadmill walking, hypercapnia was again associated with increases in f_v and \dot{V}_E . The absolute change in \dot{V}_E , however, was only half that seen in response to 5% CO₂ at rest, as V_T did not increase in response to CO₂ during exercise.

Exposure to 12% O₂/1% CO₂ under resting conditions resulted in much smaller increases in f_v and V_T than seen in response to CO₂ (Fig. 5) and only a 40% increase in \dot{V}_E . In contrast, a significant 100% increase in \dot{V}_E accompanied hypoxia during exercise. Breathing frequency rose continuously during hypoxic exercise (35.3% between 2 and 8 min) and was primarily responsible for the increase in \dot{V}_E .

Coupling of locomotor and respiratory systems was only significantly greater than the maximum which could occur due to chance during air-breathing (Fig. 6). The rate of entrainment observed during the hypoxic trials ($19.0 \pm 6.9\%$) was significantly less than that seen during air-breathing ($34.8 \pm 3.9\%$) and could possibly be accounted for by chance alone. In response to CO₂, two animals showed approximately 20% entrainment while one animal demonstrated entrainment 59% of the time. Because of this large variability, the mean level of coupling during hypercapnia ($32.8 \pm 13.0\%$) was not significantly different from either chance (20%) or air-breathing values.

Discussion

O₂ and CO₂ sensitivity during rest and exercise

As shown by Fig. 5, there were marked differences in the ventilatory responses

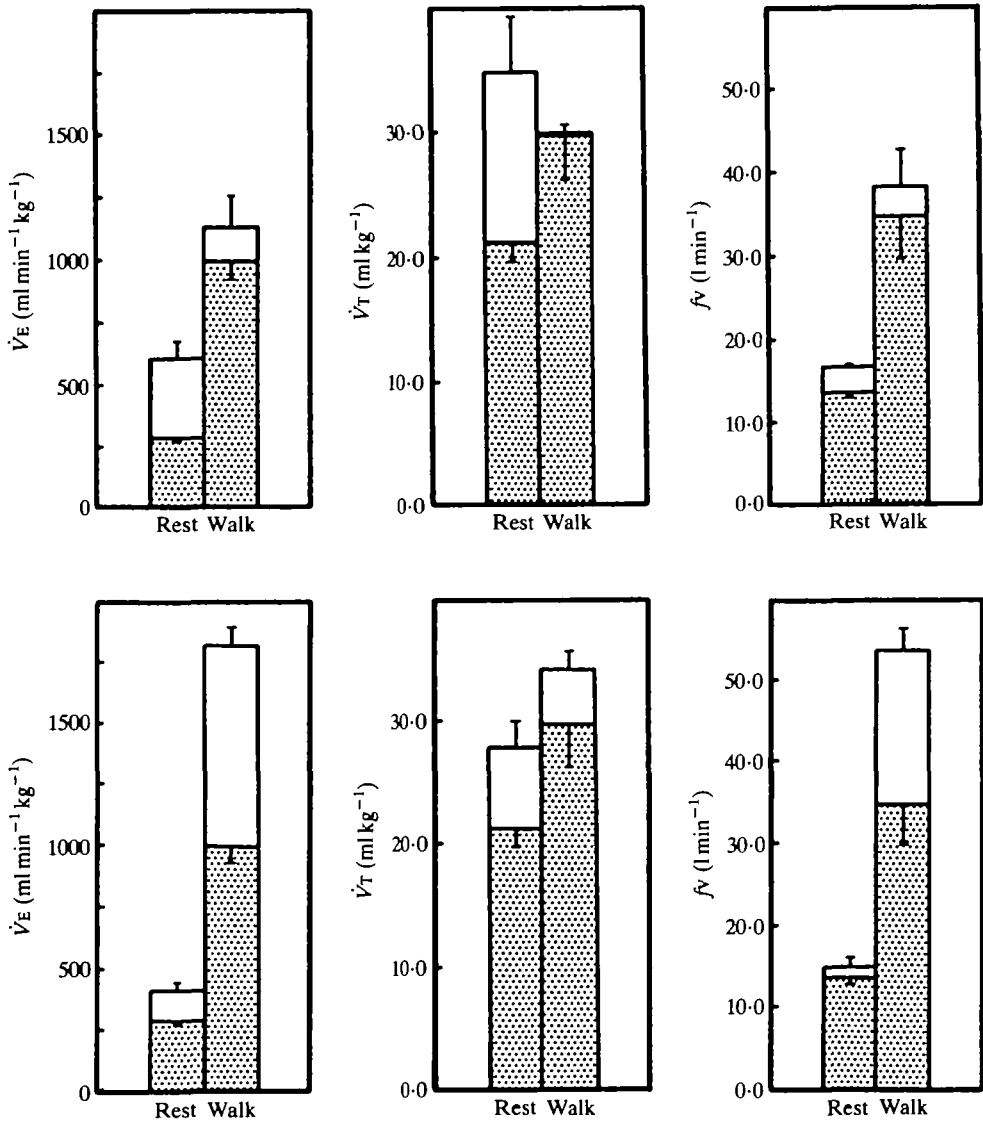


Fig. 5. Ventilatory responses to changes in inspired CO₂ and O₂ during rest and exercise. Values of \dot{V}_E , \dot{V}_T and f_v at rest ($N = 4$) and during exercise ($N = 3$) are shown during air-breathing (shaded columns) and during inspiration of 5% CO₂ (top three panels, open columns) or 12% O₂ (bottom three panels, open columns) (mean \pm s.e.). See text for further details.

to hypoxic and hypercapnic gases between rest and treadmill exercise conditions. Unfortunately, in the absence of blood gas data, it is very difficult to account for these changes. The magnitude of the ventilatory response to hypoxia appeared to be greatly enhanced during treadmill walking in geese. Increases in hypoxia sensitivity have been documented previously in the chicken (Brackenbury *et al.*

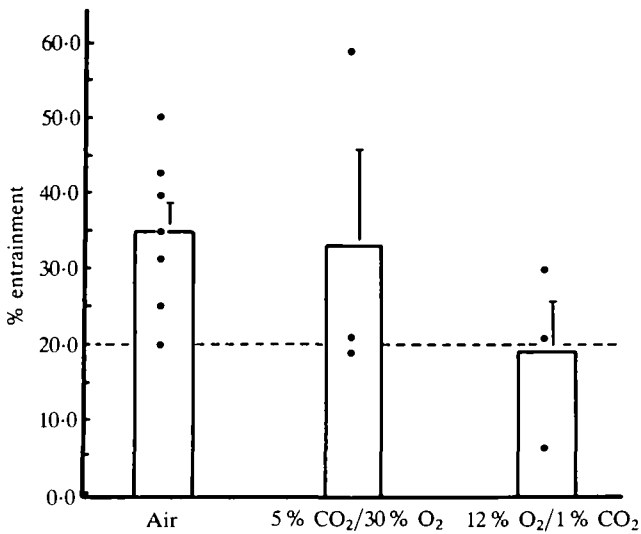


Fig. 6. Percent of total time entrained while running at 0.52 m s^{-1} breathing air (four birds, $N = 7$), 5% $\text{CO}_2/30\% \text{ O}_2$ and 12% $\text{O}_2/1\% \text{ CO}_2$ (three birds, $N = 3$). Individual data points are shown (●) as well as mean levels ($\pm \text{s.e.}$) (dashed line shows 20%, the maximum entrainment predicted by chance).

1982). Although these results may represent a state-dependent difference in hypoxic sensitivity, inspiration of 12% O_2 during exercise probably resulted in lower P_{aO_2} levels than those produced by inspiration of 12% O_2 at rest. We attempted to measure levels of arterial blood gases in the present study but this experimental procedure produced substantial changes in the respiratory responses of the geese to simple treadmill walking and the data were discarded.

In contrast to the apparent increase in hypoxic sensitivity, there appeared to be a decrease in CO_2 sensitivity of these birds during treadmill exercise (Fig. 5). Breathing frequency increased consistently during rest and exercise. However, the large increase in V_T observed during rest was not seen during treadmill walking. Conversely, Brackenbury *et al.* (1982) found no change in the ventilatory response to CO_2 during exercise in the chicken, although he did show a decrease in the response of V_T to CO_2 from rest to exercise. Without arterial partial pressures of CO_2 , however, it is difficult to assess sensitivity properly. The small sample size and the higher degree of variability of the ventilatory responses to hypoxia and hypercapnia during exercise relative to rest may also account for some of the differences.

Locomotor-respiratory entrainment

Considerable attention has been given to the relationship between locomotor and ventilatory patterns during bipedal activity (walking, running and cycling) in humans. However, there are very few data describing the relationship between stepping frequency and breathing frequency in birds. Evidence of air sac pressure

pulses related to footfall during treadmill running in ducks (Brackenbury, 1986) has been presented anecdotally, but the exact relationship between locomotor and respiratory rhythms has not been previously examined in geese.

One advantage of using birds to examine the interaction of hindlimb locomotor activity and the respiratory system, is that birds, unlike humans, are true bipeds, and show complete separation of hindlimb and forelimb function. Conversely, humans still possess quadrupedal motor programs, so that the effects of arm swing on respiratory pattern must also be considered. That this is important is shown by the observation that human entrainment rates are consistently higher during running (Bechbache & Duffin, 1977; Bramble, 1983) than during cycling (Bechbache & Duffin, 1977; Yonge & Petersen, 1983). This difference has previously been attributed to decreased visceral movement during cycling compared with running (Yonge & Petersen, 1983). In addition, entrainment of human ventilation by arm motion alone has been documented (Paterson *et al.* 1986). Decerebrate geese, however, have been shown to have entrainment rates identical to intact animals, in spite of the fact that the decerebrate geese were suspended in a sling that drastically reduced visceral movements during locomotion (Funk *et al.* 1989). This, combined with the complete separation of hindlimb and forelimb function, may make birds the animal of choice for separating hindlimb-respiratory interactions from forelimb-respiratory interactions.

The rates of coupling found in geese during treadmill walking/running were significantly greater than chance (a maximum of 34.8%), but substantially less than the maximum seen in humans during some studies of treadmill running. Although Kay *et al.* (1975) found no evidence of entrainment between locomotor and respiratory patterns during human treadmill running, Paterson *et al.* (1987) more recently found the two patterns to be coupled up to 45% of the time.

There are several factors that could account for this reduced degree of synchrony in geese. First, the use of 10-s intervals compared with less than 1-s intervals (Paterson *et al.* 1987) to calculate coupling ratios may account for some of the differences (Funk *et al.* 1989). Second, as discussed above, forelimb interactions may cause an increase in entrainment in humans. Finally, in birds, respiration, through evaporative heat loss, plays a significant role in body temperature control. Small increases in body temperature can lead to significant changes in breathing pattern, primarily through increases in breathing frequency (Brackenbury *et al.* 1981). Thus, increases in f_v as a result of changes in body temperature (T_b), could disrupt the relationship between locomotion and respiration. Collectively, these factors could account for the 10% difference in entrainment rates seen between humans and geese during treadmill running.

Effects of increasing locomotor frequency on entrainment

Treadmill

Just as the effects of limb movement rate on entrainment are not clear in humans, the present results with geese during treadmill running were not

definitive. It should be noted that, owing to the difficulty of increasing stride frequency independently of work rate and \dot{V}_E , no attempt was made to control work rate as the rate of limb movement was increased. However, this was not felt to be crucial since the increases in metabolic rate and \dot{V}_E associated with increases in treadmill velocity over this range were small. Furthermore, previous work has shown entrainment rate to be independent of the changes in metabolic rate and \dot{V}_E associated with increases in work rate in these animals (Funk *et al.* 1989).

Like the increase in entrainment observed with increasing locomotor frequency in humans (Bechbache & Duffin, 1977), increases in entrainment in geese, although not significant, were associated with increases in stride frequency over the 0.40–0.52 ms⁻¹ range. With further increases in velocity to 0.72 ms⁻¹, however, f_v increased by 30.1 % over the 8 min run and entrainment fell to 27.1 %. Panting was occasionally observed at the end of the 0.72 ms⁻¹ runs. Rectal temperature measurements replicated twice on one animal at all three velocities showed increases of 0.85°C at 0.72 ms⁻¹. Thermal hyperventilation has been associated with increases in body temperature of this magnitude during treadmill running in ducks (Kiley *et al.* 1979). Smaller temperature changes of 0.50°C, similar to those seen in geese running at 0.40 and 0.52 ms⁻¹, did not result in thermal panting in fowl (Brackenbury, 1986). Thus, although not conclusive, the data from the treadmill study suggest that increased locomotor output, whether acting *via* afferent (Iscue & Polosa, 1976) or efferent interactions (Viala *et al.* 1987), increases locomotor-respiratory coupling. However, there appears to be an apparent limit to this coupling, where further increases in velocity are associated with sufficient increases in T_b that entrainment is suppressed in favor of thermoregulation.

Free running

As seen with humans (Paterson *et al.* 1987), the degree of entrainment during overground running was significantly greater than that seen during treadmill running. Similarly, entrainment decreases when preferred cycle rhythm, chosen by the subject, is replaced with an imposed pedalling rate (Jasinskas *et al.* 1980). It is possible that the treadmill, by forcing locomotor frequency, upsets any internal rhythm that the locomotor system may prefer (Paterson *et al.* 1987), and thereby decreases entrainment.

In contrast to treadmill running, the extent to which locomotor and respiratory systems were coupled during overground running increased significantly with increasing velocity. This difference may have been due to the larger velocity range used during free running (0.40–0.72 ms⁻¹ on the treadmill *vs* 0.51–1.58 ms⁻¹ outside). However, if this were the case, it would be expected that entrainment would have shown a continuous increase as treadmill velocity increased from 0.52 to 0.72 ms⁻¹, rather than the observed decrease. Thus, as already suggested, increased thermoregulatory demands on ventilation may have been responsible for this decrease in entrainment during treadmill locomotion. During overground locomotion, although body temperature was not measured, the birds showed no

signs of thermal stress at the termination of even the fastest trials. Thermal panting was never observed. Thus, from the results presented here and the previous demonstration that entrainment is not affected by work rate in geese (Funk *et al.* 1989), it appears that if thermal stresses are reduced, increases in limb movement frequency alone enhance locomotor-respiratory coupling.

Effects of hypoxia and hypercapnia on entrainment

The prevailing ventilatory drive appears to affect the strength of locomotor-respiratory coupling. Like previous results in humans (Paterson *et al.* 1987), an increased hypoxic drive to breathe led to a reduction of entrainment to levels which could simply be due to chance in all three geese tested. Breathing frequency rose continuously over the course of the 8 min run, causing a twofold increase in \dot{V}_E relative to air-breathing levels. The reasons for the continuous rise in f_v with acute exposure to hypoxia remain unclear. However, these data suggest that the effects of increased peripheral chemoreceptor drive on breathing pattern were incompatible with the effects of the locomotor system on ventilatory pattern. Consequently entrainment was suppressed.

The effects of hypercapnia on the relationship between respiration and locomotion were less clear. Two animals showed a fall in coupling to chance levels while one showed an increase to approximately 60% entrainment. The wide variability of the entrainment data makes it very difficult to determine the effects of a hypercapnic ventilatory drive on the relationship between respiration and locomotion.

Although the data presented here provide no direct indication of the mechanisms responsible for the production of locomotor-respiratory entrainment, a number of studies suggest possible mechanisms. The synchronization of locomotor and respiratory rhythms in paralyzed, C2 spinalized rabbits (Viala *et al.* 1979) implicates spinal interactions in the development of entrainment. Locomotion may also influence respiration through central nervous system collateral projections at the brainstem level. This is supported by the feedforward effects of the subthalamic and the mesencephalic locomotor regions on respiration (Eldridge *et al.* 1985) as well as the development of entrainment in decorticate, paralyzed rabbits (Viala *et al.* 1987). However, the strong effects on ventilatory pattern due to electrical stimulation of muscle (Cross *et al.* 1982), limb afferents (Kalia *et al.* 1972) and ventral roots to limb muscles (Waldrop *et al.* 1986) suggest a role for afferent feedback in entrainment as well. The partial entrainment of respiration by limb and cutaneous afferent stimulation in anesthetized cats (Iscoc & Polosa, 1976) provides further support. Thus, it seems likely that afferent and efferent locomotor input, following one or several levels of integration, produce entrainment through a collective interaction on some portion of the respiratory pattern oscillator (Paterson *et al.* 1987).

In accordance with this idea, the frequency of locomotor-respiratory coupling during walking/running in geese is markedly enhanced by increases in limb movement rate. The various inputs affecting ventilatory pattern also appear to be

arranged in a hierarchy, with locomotor entrainment near the bottom. Under certain conditions the influence of locomotor pattern on respiration is expressed, but any additional ventilatory stimuli, especially those that mediate their effects through an increase in breathing frequency, such as increased body temperature or hypoxia, appear to override the entraining effects of locomotor pattern on respiratory rhythm and entrainment is either reduced or suppressed.

In conclusion, although there is conflicting evidence as to the effects of velocity on locomotor-respiratory coupling, an increase in coupling with velocity during overground running in geese has been clearly demonstrated. It is suggested that these increases in entrainment are not due to increases in metabolic rate, but to increases in limb movement frequency alone. Variations in experimental design may account for much of the current confusion surrounding the effects of increased velocity on entrainment, as there are significant differences in the degree of entrainment observed during treadmill and overground running.

Ventilatory drive has also been shown to affect the strength of coupling between locomotor and respiratory systems. Ventilatory stimuli such as hypoxia and elevated body temperature appear to reduce or override the effects of locomotion on ventilatory pattern so that entrainment is diminished.

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