ROLE OF THE TELENCEPHALON IN THE SYNCHRONIZATION OF LOCOMOTOR AND RESPIRATORY FREQUENCIES DURING WALKING IN CANADA GEESE

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Summary

To elucidate the importance of telencephalic structures and the effects of metabolic rate in the production of locomotor–respiratory coupling, we examined the relationship between locomotor and ventilatory patterns in: (1) intact trained geese, and (2) brainstem-stimulated (medullary reticular formation) decerebrate geese, that were walking on a treadmill. The decerebrate geese, however, were not completely self supporting. Thus, while the two groups walked with similar stride frequencies (fs), they did so at two different work rates.

While at rest, tidal volume (V_T), breathing frequency (f_V) and minute ventilation ($\dot{V}_E$) were very similar in the two groups. $\dot{V}_E$ increased 120 % during walking in the intact geese, primarily as a result of increases in f_V, while both V_T and f_V increased to produce a smaller 40 % increase in $\dot{V}_E$ in the decerebrate birds. Although the magnitude of the increase in $\dot{V}_E$ was three times greater in the intact geese, the relationships between $\dot{V}_E$ and oxygen uptake ($\dot{V}_{O_2}$) and $\dot{V}_E$ and CO_2 output ($\dot{V}_{CO_2}$) were similar in the two groups.

Significant coupling between locomotor and respiratory patterns was found in both intact (28·3 %) and decerebrate birds (28·9 %), suggesting that the telencephalon is not essential for the coupling of locomotor and respiratory rhythms during walking in geese. In addition, the incidence of locomotor–respiratory synchrony was virtually identical in the two groups in spite of a threefold difference in metabolic work rate.

Introduction

The locomotor and respiratory systems are sometimes very tightly coupled during locomotion in a variety of animals, especially those which display synchronous (in phase) movements of the limbs (birds in flight: Hart & Roy, 1966; rabbits hopping: Viala *et al.* 1987; wallabies bounding: Baudinette *et al.* 1987; horses galloping: Bramble & Carrier, 1983). The relationship between breathing

pattern and bipedal locomotion employing alternating (out of phase) movements of the limbs is much less obvious. Several studies using humans performing bicycle (Kay *et al.* 1975; Bechbache & Duffin, 1977; Jasinskas *et al.* 1980; Kohl *et al.* 1981; Yonge & Petersen, 1983; Paterson *et al.* 1986) and running exercise (Kay *et al.* 1975; Bechbache & Duffin, 1977; Bramble, 1983; Paterson *et al.* 1987) have yielded conflicting results. Thus, although Kay *et al.* (1975) failed to show a relationship between respiratory and locomotor cycles during treadmill walking, more recent work suggests that respiratory and locomotor cycles may be coupled up to 80 % of the time during unrestrained, overground human running (Bramble, 1983; Paterson *et al.* 1987). Furthermore, some studies have shown increases in entrainment with increased work rate (Jasinskas *et al.* 1980) or limb movement rate (Bechbache & Duffin, 1977) while others have shown neither (Kohl *et al.* 1981). Part of this confusion may be the result of the failure to examine independently the effects of metabolic rate and limb movement rate on entrainment (Bramble & Carrier, 1983).

It has been suggested that mechanical interactions between the locomotor musculature and the respiratory system force a coupling of the two systems (Bramble & Carrier, 1983). Alternatively, recent experiments on paralyzed rabbits suggest that locomotor–respiratory coupling is produced *via* a central neural circuit (Viala *et al.* 1987). Somatic afferent feedback also appears to be involved in the production of this entrainment (Iscoe & Polosa, 1976). In addition to these factors, the greater levels of entrainment observed in trained athletes relative to non-athletes (Bramble, 1983) and the increase in entrainment associated with audible pacing cues (Bechbache & Duffin, 1977; Yonge & Petersen, 1983) suggest that telencephalic influences may be partly responsible for the coupling of locomotor and respiratory patterns.

It has been shown in a wide variety of species that, following decerebration, the vertebrate brainstem is capable of producing normal locomotor (McClellan, 1986) and respiratory behaviour (Feldman *et al.* 1988). Thus, the first purpose of this study was to determine whether locomotor–respiratory interactions at the level of the brainstem are sufficient to produce entrainment of the two motor patterns or whether contributions from the telencephalon are required for the production of entrainment.

To this end, we compared the degree of entrainment found in a group of intact, trained walking geese with that seen in a group of decerebrate, brainstem-stimulated walking geese. Since general anesthesia greatly depresses locomotor as well as respiratory function, these experiments could not be performed on anesthetized animals. Surgical removal of all brain regions rostral to the brainstem (the telencephalon in birds) produces a decerebrate animal that is incapable of any perception of pain and, therefore, does not require the subsequent use of general anesthetics (Loeser & Black, 1975; Wall, 1975; Adams, 1980). Unanesthetized decerebrate animals are capable of a wide range of spontaneous and stimulated motor behaviour (e.g. breathing and walking: Sherrington, 1910, 1915; McClellan, 1986; Feldman *et al.* 1988). This type of experimental preparation is the only way

to examine the relationship between locomotor and respiratory patterns in the absence of telencephalic influences humanely without the confounding effects of general anesthetics.

The second purpose of this study was to examine the effects of changes in metabolic rate on entrainment regardless of changes in locomotor frequency. By supporting the decerebrate birds in a sling overlying the treadmill and using low-intensity electrical stimulation to induce walking movements, it was possible to produce and sustain locomotion without the associated large increases in meta-bolic rate normally seen during walking in intact birds. Thus, by matching the walking speeds (i.e. stride frequencies) of the intact geese with those of the decerebrate birds, we were able to examine the relationship between locomotor and respiratory patterns where similar stride frequencies were produced at markedly different metabolic work rates.

Geese were our animal of choice because the neural control of bipedal walking in geese is very similar to that of man (Sholomenko & Steeves, 1987; Steeves *et al.* 1987), yet they are 'true' bipeds and thus have complete separation of forelimb and hindlimb function. Humans, however, still possess quadrupedal motor programs and arm swing has been shown to entrain ventilation in humans (Paterson *et al.* 1986). Thus, in walking birds such as geese, forelimb–respiratory interactions are minimized and a more direct examination of the role hindlimb–respiratory interactions play in the development of entrainment is possible.

Materials and methods

Intact geese

Training and equipment

Four Canada geese (*Branta canadensis*; 4·2 ± 0·2 kg) were raised from hatching and imprinted upon one of the authors (GDF). The goslings spent at least 4 h per day with the 'foster parent' for their first 3 weeks, 1 h of which was spent wearing a facemask that formed an airtight seal caudal to the nares and beak opening. From 3 weeks to 3 months of age, they spent at least 2 h per day with the surrogate parent in daily walks with the same masks in place. Thus as the birds increased in size, the contribution of the mask to overall dead space volume decreased in both absolute and relative terms. In addition, each gosling was run on the treadmill at least once a week to become accustomed to the laboratory environment.

At 3 months of age more intensive treadmill training was started. First, the birds were fitted with a slightly heavier (60 g), form-fitting mask. A port (10 mm inside diameter) on the dorsal surface of the mask, overlying the nares, allowed easy attachment and removal of a pneumotachograph (Fleisch model 00) to monitor ventilation. The mask and pneumotachograph had a combined dead space volume of 4·0 ml and therefore increased dead space by approximately 10 % (Fedde *et al.* 1986). The birds became accustomed to the new mask after less than 1 week of daily training runs (10–15 min duration; 0·30–0·72 m s^{-1}). However, an additional

A

Pneumotachograph ($\dot{V}$E, VT, fv)

To gas analyzers
(PECO$_2$, PEO$_2$)

Mask

Force plate (fs)

B

Mask

To gas analyzers
($\dot{V}$CO$_2$, $\dot{V}$O$_2$)

Douglas bag

Flow meters

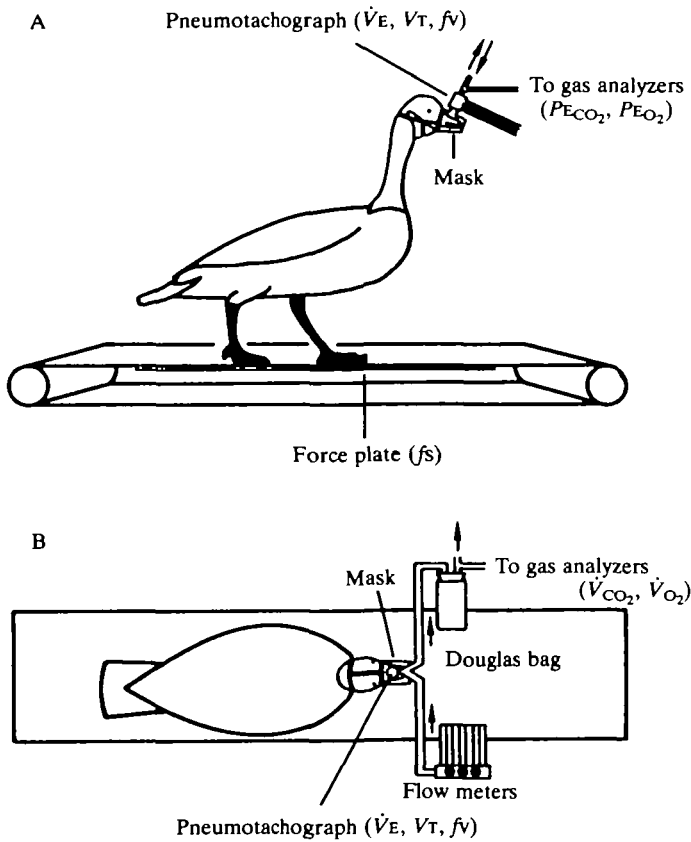Pneumotachograph ($\dot{V}$E, VT, fv)

Fig. 1. Schematic diagram illustrating the experimental arrangement for monitoring (A) mixed expired gas concentrations and (B) metabolic rate in the intact geese. See text for further details. PECO$_2$, mixed expired CO$_2$; PEO$_2$, mixed expired O$_2$; $\dot{V}$E, minute ventilation; VT, tidal volume; fv, breathing frequency; fs, stride frequency; $\dot{V}$CO$_2$, CO$_2$ output; $\dot{V}$O$_2$, O$_2$ uptake. Arrows indicate direction of air flow.

8 weeks of training was required before the birds would run calmly with the pneumotachograph and associated equipment attached to the mask.

The pneumotachograph was attached to a differential pressure transducer (Validyne DP 103-18) and an integrating amplifier (Gould) to obtain tidal volume (VT), breathing frequency (fv) and minute ventilation ($\dot{V}$E). The pneumotachograph was heated to prevent condensation. Calibration was achieved 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} (Fig. 1A). Oxygen uptake ($\dot{V}$O$_2$) and CO$_2$ output ($\dot{V}$CO$_2$) were measured by passing 10 l of air per minute past the end of the pneumotachograph through lightweight anesthetic tubes and collecting the effluent gas in a 2 l Douglas bag (Fig. 1B). The

Douglas bag was then emptied and 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. Each measurement took approximately 30 s and could not be performed while mixed expired gas measurements were being taken. Thus, two runs were carried out for each bird, one during which mixed expired gas concentrations were measured and one during which $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were measured (except for bird no. 1 which could not be trained to wear the gas delivery system while walking).

The birds were run in a 37 cm wide × 80 cm long × 60 cm high plywood box overlying a variable-speed, motor-driven treadmill. A window was cut in the front of the box, since this facilitated their willingness to walk. The treadmill belt lay over a force plate constructed from a 7 cm × 15 cm piece of piezoelectric film sandwiched between two insulating pieces of paper cemented to the back of a 31 cm × 60 cm piece of 3·2 mm mild steel. The signal from the force plate, indicating footfall, was amplified and recorded on an electrostatic recorder (Gould ES1000 B).

Protocol

Each bird was fitted with the ported mask and placed on the treadmill. They were allowed at least 5 min before the pneumotachograph and either the intake line to the gas analyzers (Fig. 1A) or the air delivery system was attached to the mask (Fig. 1B). Pre-locomotion data, including $\dot{V}_E$, V_T, fv and mixed expired gas concentrations ($P_{E_{O_2}}$ and $P_{E_{CO_2}}$) or $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, were recorded for 1 min before the treadmill was turned on. Treadmill velocity was increased gradually from 0 to $0·40 \, \text{m s}^{-1}$ over 2 s to prevent startling the bird. This particular velocity was chosen to match the step frequency of intact birds to that of decerebrate birds (which, in turn, was a function of stimulation intensity; see section on decerebrate animals). Each run lasted 8 min. Recovery was followed for 5 min only, since the birds had a tendency to struggle if this period was extended. Animals were not run more than once per day during data collection and only trials free of disturbance were included in the data analysis.

Measurements

Resting measurements of $\dot{V}_E$, V_T, fv, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and R ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) represent means (±s.e.) of 21 measurements on four animals, with the exception of $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and R which represent means (±s.e.) of nine measurements on three animals. Walking values of $\dot{V}_E$, V_T and fv represent means (±s.e.) of seven trials on four animals and values of $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and R represent means (±s.e.) of three trials on three geese.

Following completion of the preceding measurements, rectal temperature was monitored through walking and recovery in one animal on two separate days. Arterial blood gas tensions and pH were measured in one animal using cannulation, sampling and analysis procedures described below for decerebrate geese. Similar measurements were attempted on the three remaining intact geese.

However, the procedures greatly altered the ventilatory responses of these birds and these data were rejected.

Decerebrate animals

All surgical procedures were performed under general anesthesia (induction level, 3 % halothane/20 % nitrous oxide; maintenance level, 1–2 % halothane/ 20 % nitrous oxide; both on a background of 95 % O_2/5 % CO_2). The right brachial vein was cannulated for infusion of fluids when necessary and the right brachial artery was cannulated for monitoring blood pressure and arterial blood sampling. Blood samples of 0·6 ml were taken and the catheter was flushed with saline containing 100 i.u. ml^{-1} of heparin. Blood samples were then stored on ice until they could be analyzed for arterial CO_2 tension (Pa_{CO_2}), arterial oxygen tension (Pa_{O_2}) and arterial pH (pHa) using electrodes (Radiometer BMS-Systems, Copenhagen, Denmark) thermostatted to body temperature (41°C). The blood gas electrodes were calibrated with humidified gas mixtures delivered by a mixing pump (Radiometer GMA2 gas-mixing pump) and the pH electrode was calibrated using precision buffer solutions (Radiometer).

A tracheostomy was then performed to allow continued administration of general anesthetic and subsequent monitoring of ventilation *via* pneumotachography, as described above. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were measured as described previously, except a 10 l Douglas bag was used for air collection. 30 ml air samples were taken from the collection bag using glass, leuer-lock syringes. These were later injected into the Beckman gas analyzers to determine the fractional concentrations of O_2 and CO_2 in the effluent gas. A temperature probe was placed approximately 25 cm down the esophagus to monitor body temperature.

The animal was then suspended in a sling overlying the treadmill belt. The bird's head was placed in a stereotaxic headholder and it was decerebrated as previously described by Steeves *et al.* (1987). All incision sites and stereotaxic headholder pressure points were generously infiltrated with local anesthetic (2 % xylocaine) throughout the course of surgery and during all subsequent experimental trials. Following decerebration, electromyograph (EMG) electrodes were implanted percutaneously in the iliotibialis cranialis (ITC) muscles (synonymous with mammalian sartorius muscles). General anesthesia was then discontinued and the birds were allowed a minimum of 1·5 h recovery to remove all effects of the anesthetic before electrical brainstem stimulation was initiated (complete washout of halothane takes 20–30 min following up to 6 h of surgery in man; *Compendium of Pharmaceuticals and Specialties*, 1982). Stride frequency (*f*s), defined as the number of complete step cycles performed by the right or left leg per minute, was obtained from EMG signals which were amplified and filtered prior to storage on an electrostatic recorder.

Brainstem stimulation (pulse duration, 2 ms; pulse frequency, 60 Hz; stimulation intensity range, 30–120 μA) was carried out on nine Canada geese (3·6 ± 0·2 kg) using procedures described by Steeves *et al.* (1987). High-intensity stimulation (100 μA) was used to localize the stimulation site. Stimulation intensity

was then decreased to zero and gradually increased to establish the current threshold intensity necessary to evoke walking. Typically, it took between 30 and 60 min to establish optimal electrode position and current threshold for evoked locomotion (Steeves *et al.* 1987); thus, anesthetic had been removed for a minimum of 2 h before any data were collected. During higher-intensity stimulation, the animals generated sufficient force during walking to be completely self supporting. However, at the lower stimulation intensities used here during data collection, the sling supported a large portion of the animal's weight, enabling the decerebrate animal to generate locomotor patterns of similar frequency and timing to intact birds at much lower metabolic work rates.

Ten minute locomotion trials were then initiated at current stimulation intensities 20 % greater than threshold. This level of stimulation produced stepping of a specific frequency immediately with the onset of brainstem stimulation and avoided the delay often associated with stimulation at threshold current strengths. The use of low stimulation intensities made it possible to produce walking for periods greater than 10 min. By increasing brainstem stimulation intensity, *f*s, stride force and work rate could all be increased. Since another purpose of this study was to examine the effects of work rate on the relationship between locomotor and breathing patterns, however, the lower stimulation intensities were used to maximize the metabolic work rate difference between decerebrate and intact groups walking at similar stride frequencies. Stimulation sites were identified as described by Steeves *et al.* (1987). Values of $\dot{V}_E$, V_T and *f*v represent means ($\pm$s.e.) of 13 runs on nine animals and values of $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and R represent means ($\pm$s.e.) of nine trials on nine animals.

Protocol

$\dot{V}_E$, V_T and *f*v were recorded for 2 min prior to stimulation, during 10 min of brainstem-evoked walking at a speed of $0.40 \, \text{m s}^{-1}$ and for 15 min of post-stimulation recovery. Arterial blood samples and body temperature measurements were taken 30 s before stimulation, and at 1, 2, 4, 6 and 8 min of evoked walking and 5, 10 and 15 min of recovery. Effluent gas samples for determining $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were taken 30 s before stimulation, and at 1, 6 and 10 min of evoked walking and 5, 10 and 15 min of recovery. Stride frequency was recorded throughout the brainstem-stimulated walking period. To avoid any transient increase in $\dot{V}_E$ associated with switching the treadmill on and off, all measurements, from pre-stimulation to post-stimulation, were made with the treadmill on.

To ensure that all effects of the general anesthetic had worn off and that the animals had stabilized following surgery, four animals were run twice (hence 13 runs on nine birds). The first run, as described above, took place a minimum of 2 h following the removal of the general anesthetic. The second run was performed 2 h following the first; 4 h after removal of the general anesthetic. There were no significant differences between the responses seen at 2 and 4 h. Thus, it was felt that the birds had stabilized by 2 h and that sufficient time had been allowed for complete removal of general anesthetic.

Data analysis

Entrainment has been defined mathematically as a close integer or half-integer relationship between stride frequency (fs) and breathing frequency (fv) (Pavlidis, 1973). Assuming random association of fs and fv and assigning confidence limits of ± 0.05 for entrained fs/fv values, chance coupling of locomotor and breathing patterns could reach 20 % (Paterson *et al.* 1987). To determine the degree of entrainment, coupling ratios (fs/fv) were calculated for each 10 s interval throughout the locomotor periods of both intact and decerebrate birds.

To determine the importance of the sampling interval duration on calculated entrainment rates, coupling ratios were also calculated on the basis of 2 s intervals in one animal. On the few occasions when breathing rates exceeded $30\,\mathrm{min}^{-1}$ (i.e. each breath lasted <2 s) in this animal, coupling ratios were calculated on a breath by breath basis. Entrainment rates calculated using 2 s intervals were 2·5 % greater than when calculated using 10 s intervals. Thus, although analysis based on 10 s periods will lead to slightly conservative values of entrainment relative to shorter sampling intervals (e.g. <1 s, Paterson *et al.* 1987), it ensured that, for any coupling of fs to fv to be counted as entrainment, it had to be sustained for at least 10 s (approximately six strides or 12 consecutive steps). After normalizing the percentage entrainment data (arcsin transformation), a *t*-test was used to ascertain whether the percentage of entrainment was significantly greater than chance (i.e. 20 %). Although it was difficult to determine exactly how long it took for the birds to come to a new steady state following the onset of exercise, from Fig. 2 and from previous work with birds (Brackenbury *et al.* 1981*a*) it appeared that new 'steady states' were reached within 4 min. Thus, ventilatory data were averaged over the final 4 min of each walking period. ANOVA and Tukey's multiple comparison test were used to test the difference between means of all respiratory and entrainment data. Regression analysis and ANCOVA were used to test for differences in the relationship between $\dot{V}\text{E}$ and $\dot{V}_{O_2}$ and $\dot{V}\text{E}$ and $\dot{V}_{CO_2}$ in both intact and decerebrate groups. Values of P greater than 0·05 were assumed to be significant.

Results

All effective locomotor electrical stimulation sites fell within the areas described by Steeves *et al.* (1987; fig. 1, P 3·00). They were histologically verified within the mid-medulla, between the nucleus dorsalis pars centralis (Cnd), ventromedially, and the nucleus and tract of the trigeminal nucleus (TTD), dorsolaterally. The stimulation sites extended caudally from 300 μm caudal to the caudal extent of the twelfth cranial nerve nucleus, and rostrally to the rostral extent of the twelfth nerve nucleus. It should be noted that with the onset of brainstem stimulation in the decerebrate animals, three different transient ventilatory responses, lasting less than 20 s, were observed. Three animals stimulated within the TTD/Cnd region, 150–300 μm caudal to the caudal extent of the twelfth cranial nerve nucleus, showed immediate decreases in $\dot{V}\text{E}$ ($>400\,\mathrm{ml\,min}^{-1}$) at the onset of stimulation. In contrast, the three animals stimulated within the TTD/Cnd region,

300–600 μm rostral to the caudal margin of the twelfth nerve nucleus showed immediate increases in $\dot{V}_E$ (>800 ml min^{-1}). The final three animals stimulated in TTD/Cnd, between the two stimulation sites discussed above, showed small changes in $\dot{V}_E$ (<200 ml min^{-1}); one animal exhibited an increase, one showed a decrease and the other showed no change. Aside from these transient differences in the ventilatory responses associated with the onset of electrical stimulation, the overall responses of the decerebrate animals were very similar.

The pre-walking and steady-state walking values of the various respiratory variables monitored are shown in Table 1 for both intact and decerebrate geese. Pre-walking tidal volumes (V_T) were very similar between the two groups, but a slightly larger f_V in the intact animals led to a larger 'resting' $\dot{V}_E$ in this group. Similarly, $\dot{V}_{O_2}$ was slightly elevated in the intact animals relative to the decerebrate birds at rest. $\dot{V}_{CO_2}$ was significantly greater in the intact animals. These differences were probably a consequence of a greater reliance on fat metabolism (R = 0.7) by the decerebrate group, owing to a 12 h pre-surgery starvation period, *versus* carbohydrate utilization (R = 1.0) by the non-starved intact birds (Schmidt-Nielsen, 1979).

With the onset of walking, $\dot{V}_E$ increased significantly in both intact and decerebrate groups (Fig. 2). However, there was a small overshoot in $\dot{V}_E$ in the intact animals at the start of walking that was not normally seen in the decerebrate animals. The overall magnitude of the ventilatory responses, as well as the changes in breathing pattern, were quite different in the two groups. Both V_T and f_V increased marginally during evoked walking in the decerebrate birds to produce a small but significant 40 % increase in $\dot{V}_E$. In the intact birds, however, the much larger increase in $\dot{V}_E$ (120 %) was entirely due to a 137 % increase in f_V. V_T, following an initial increase, decreased marginally over the course of the 8 min walking trial for the intact birds.

The increase in $\dot{V}_{O_2}$ between 'rest' and walking was also three times greater in the intact than in the decerebrate geese. $\dot{V}_{O_2}$ increased 142 % from 13.5 to 32.7 ml min^{-1} kg^{-1} in the intact geese, but it only increased 46.2 % from 11.3 to 15.9 ml min^{-1} kg^{-1} in the decerebrate geese. Despite the large differences in the overall magnitude of the $\dot{V}_E$ and $\dot{V}_{O_2}$ responses of the two groups to walking and the resultant reduced range over which the $\dot{V}_E/\dot{V}_{O_2}$ regression was calculated for decerebrate birds, the slopes and y-intercepts of the relationships between $\dot{V}_E$ and $\dot{V}_{O_2}$ were not different between the two groups (Table 1, Fig. 3). Similarly, the relationship between $\dot{V}_E$ and $\dot{V}_{CO_2}$ was not significantly different between intact and decerebrate birds (Table 1, Fig. 3).

Despite a transient increase in Pa_{O_2} of 0.67 kPa (5.0 mmHg), a decrease in Pa_{CO_2} of 0.4 kPa (3.0 mmHg), and an increase in pHa of 0.05 units over the first minute of evoked locomotion in the decerebrate geese (Fig. 4), blood gases, pHa and body temperature (T_b) did not change significantly between rest and the final 4 min of decerebrate walking (Table 1). Pa_{O_2} only increased by 0.2 kPa (1.5 mmHg), Pa_{CO_2} decreased by 0.13 kPa (1.0 mmHg), pHa increased by 0.04 units and T_b also increased marginally (0.2 °C) from rest to exercise. Blood gas and T_b measure-

Table 1. *Respiratory values in decerebrate and intact Canada geese recorded prior to and over the last 4 min of a 10 min (decerebrate) and 8 min (intact) walking period*

	Decerebrate					Intact				
	Rest	N	Exercise	N	% Change	Rest	N	Exercise	N	% Change
$\dot{V}_E$ (ml min^{-1} kg^{-1})	314 ± 28	13	446 ± 27†	13	42.2	417 ± 30	21	887 ± 65*†	7	119
V_T (ml kg^{-1})	29.9 ± 1.5	13	34.1 ± 2.3	13	14.6	28.2 ± 1.3	21	26.5 ± 2.4	7	-4.6
f_V (min^{-1})	10.7 ± 1.0	13	13.4 ± 0.8	13	25.2	14.8 ± 0.80	21	34.9 ± 3.0*†	7	137
f_S (min^{-1})	—	—	57.5 ± 5.0	13	—	—	—	55.2 ± 2.3	7	—
$\dot{V}_{O_2}$ (ml min^{-1} kg^{-1})	11.3 ± 0.8	8	15.9 ± 1.1†	9	46.2	13.5 ± 1.5	9	32.7 ± 3.8*†	3	142
$\dot{V}_{CO_2}$	7.6 ± 0.7	8	12.6 ± 0.6†	9	68.3	13.1 ± 1.3*	9	29.9 ± 3.8*†	3	128
R	0.67 ± 0.03	8	0.80 ± 0.03	9	—	0.98 ± 0.04*	9	0.91 ± 0.03	3	—
T_b (°C)	40.4 ± 0.7	5	40.6 ± 0.6	5	—	42.3	2	42.7	2	—
Entrainment (%)	—	—	28.9 ± 3.3	9	—	—	—	28.3 ± 4.0	7	—
Slope $\dot{V}_E/\dot{V}_{O_2}$	24.43		$r = 0.787$			18.48		$r = 0.985$		
Slope $\dot{V}_E/\dot{V}_{CO_2}$	24.74		$r = 0.988$			18.67		$r = 0.982$		
Pa_{O_2} (kPa)	12.45 ± 0.95	8	12.65 ± 0.97	8	—	11.86	1	13.73	1	—
Pa_{CO_2} (kPa)	3.32 ± 0.16	12	3.19 ± 0.12	12	—	3.67	1	3.27	1	—
pHa	7.50 ± 0.02	12	7.54 ± 0.02	12	—	7.52	1	7.56	1	—

Values are mean ± S.E., N = number of experiments.
% Change; percentage change from rest to exercise.
r, Pearson's correlation coefficient.
* Significant difference between the two groups during rest or exercise.
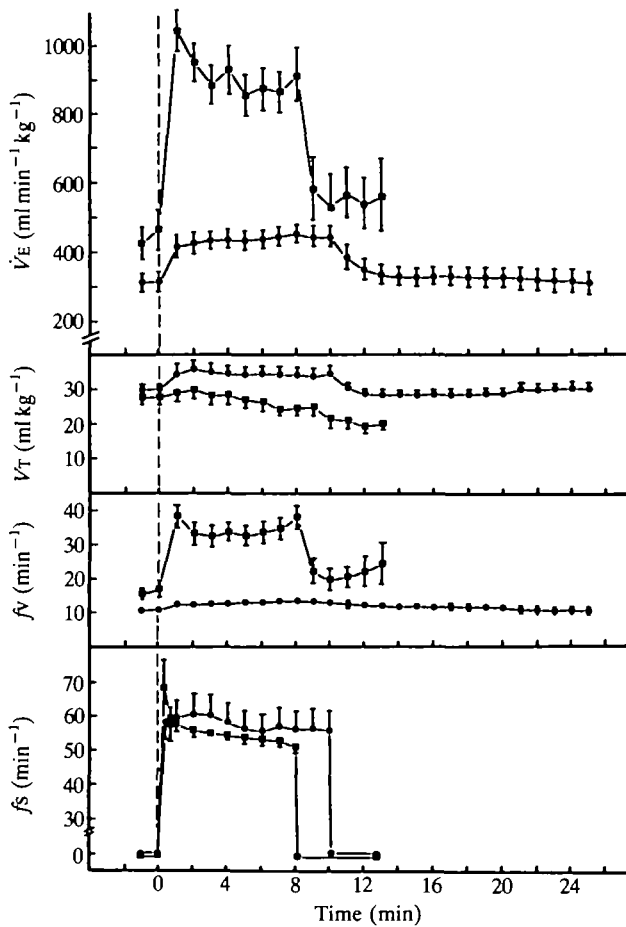† Significant difference between rest and exercise within a group.

Fig. 2. Effect of exercise on minute ventilation ($\dot{V}_E$), tidal volume (V_T), breathing frequency (f_V) and stride frequency (f_S). Walking was initiated at $t = 0$ and terminated at $t = 10$ min for the decerebrate geese (●) and at $t = 8$ min for the intact geese (■). Values are mean ± S.E.

ments were only possible in one intact animal, making comparisons between intact and decerebrate geese difficult. Arterial pH also increased by 0·04 units in the intact animals. However, Pa_{O_2}, Pa_{CO_2} and T_b showed much larger changes in the intact bird than in the decerebrate geese. Pa_{O_2} increased by 1·87 kPa (14·0 mmHg), Pa_{CO_2} decreased by 0·4 kPa (3·0 mmHg) and T_b increased by 0·4°C.

Locomotor and respiratory rhythms were coupled significantly more than the maximum predicted by chance (20 %; Paterson *et al.* 1987) in both groups. There was no significant variation in the degree of entrainment over the course of an 8 min walking trial and the coupling was always subharmonic. Since the stride frequency was the same in both groups but the level of f_V was higher in the intact animals, the coupling ratios (f_S/f_V) in the intact group were much lower than in decerebrate birds. Values of 3 and 4 steps breath^{-1} (i.e. $f_S/f_V = 1·5$ and 2·0,
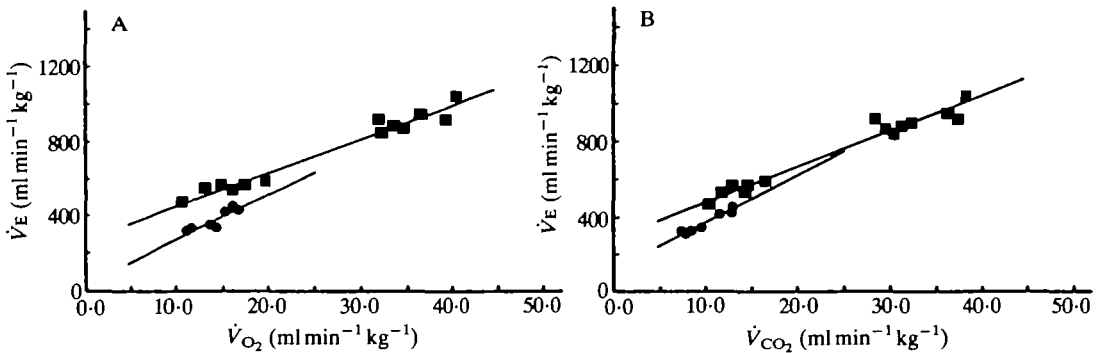
Fig. 3. Relationship between minute ventilation ($\dot{V}E$) and oxygen uptake ($\dot{V}_{O_2}$) and $\dot{V}E$ and CO_2 output ($\dot{V}_{CO_2}$) in decerebrate (●) and intact (■) geese. Each point represents the mean of nine and seven trials in decerebrate and intact geese, respectively. Values from resting and walking birds are included.

respectively) were most commonly found in the intact geese, ranging from 2 to 6 steps breath^{-1}. The coupling ratios were consistently larger at the onset of walking, declining smoothly as fv increased over time. In decerebrate birds, values ranged from 6 to 18 steps breath^{-1}, with the most commonly observed values varying between 6 and 10 steps breath^{-1} (i.e. $fs/fv = 3–5$). The highest values occurred at the very beginning of walking in the three animals that showed large, transient decreases in $\dot{V}E$ and fv at the onset of stimulation. Since fv quickly recovered in these birds, the coupling values also rapidly returned to between 6 and 10 steps breath^{-1}. Coupling values in the remaining six decerebrate geese, as seen in intact geese, were also highest at the onset of locomotion and declined very gradually as fv increased slightly over the course of the walking period. In spite of the differences in breathing pattern responses of the two groups and the resultant differences in fs/fv values, the degree of locomotor–respiratory coupling in intact ($28\cdot3 \pm 4\cdot0\,\%$) and decerebrate ($28\cdot9 \pm 3\cdot3\,\%$) geese was virtually identical.

Discussion

Ventilation during treadmill walking in intact birds

The ventilatory responses of birds to treadmill running appear to be dependent on both work rate (as reflected in this and other studies by changes in treadmill velocity: Kiley *et al.* 1979; Brackenbury *et al.* 1981*a*, 1982; Nomoto *et al.* 1983; Brackenbury, 1986) and changes in body temperature (T_b) (Kiley *et al.* 1979; Brackenbury *et al.* 1981*a,b*, 1982; Nomoto *et al.* 1983; Brackenbury & Gleeson, 1983). Similar to the present results, during submaximal exercise at ambient temperatures near 20°C, $\dot{V}E$ typically increases between two and four times, depending on velocity, in response to treadmill running in both the chicken (Brackenbury *et al.* 1982; Brackenbury & Gleeson, 1983) and the duck (Kiley *et al.* 1979, 1982). As shown by others, increases in fv are primarily responsible for the
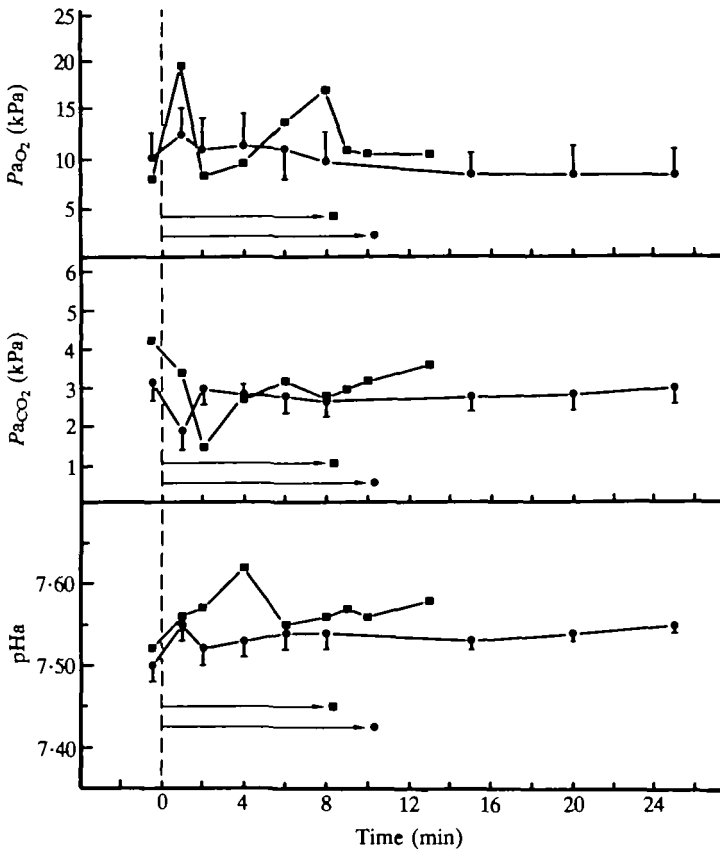
Fig. 4. Effect of exercise on Pa_{O_2}, Pa_{CO_2} and pHa. Arrows indicate duration of the walking period in decerebrate (●) and intact (■) geese. Values are mean ± S.E. (See Table 1 for N values.)

initial rise in $\dot{V}_E$ at the onset of exercise (Brackenbury *et al.* 1982; Kiley *et al.* 1982; Brackenbury & Gleeson, 1983; Brackenbury, 1986), although V_T has also been shown to increase significantly at the onset of exercise in some studies (Brackenbury *et al.* 1982; Brackenbury, 1986).

Regardless of the initial response to exercise, it is clear that, as treadmill walking progresses and T_b rises, V_T gradually decreases and fv rises, contributing more and more to the increase in $\dot{V}_E$ in the geese, as has also been shown for chickens (Brackenbury *et al.* 1982) and ducks (Kiley *et al.* 1979). If T_b increases sufficiently, thermal panting is commonly observed (Kiley *et al.* 1979; Brackenbury *et al.* 1981a) and fv can reach levels greater than 150 breaths min^{-1} (Kiley *et al.* 1979). When body temperature fluctuations are minimized (i.e. maintained below 0·5°C; Brackenbury, 1986), by using lower treadmill speeds and reducing metabolic heat production (fig. 2; Brackenbury, 1986), decreasing ambient temperature (Kiley

et al. 1982; Brackenbury, 1986) or artificially cooling the animals (Brackenbury & Gleeson, 1983), fv seldom increases beyond 55 breaths $\min^{-1}$.

Effects of decerebration on locomotion and ventilation

Although there are extensive neurological differences between intact and decerebrate geese, it has been shown for a large array of species that locomotion in decerebrate animals, whether produced spontaneously or by electrical/chemical stimulation, is similar to locomotion in intact animals (Grillner, 1975; McClellan, 1986; Steeves *et al.* 1987). The step cycle of the individual limb resembles that of the intact animal, with regard to the duration and the amplitudes of the movements at different speeds as well as EMG pattern (Grillner, 1975; McClellan, 1986; Steeves *et al.* 1987). In the case of electrical stimulation, the force generated by the limbs increases with increasing stimulation intensity, as does the speed of walking. These findings hold true for the bird as well (Sholomenko & Steeves, 1987).

Similarly, with respect to respiration, the essential neural networks responsible for the production of the normal respiratory pattern have long been known to be contained within the brainstem (Lumsden, 1923). The question remains, however, as to whether the ventilatory responses to exercise are the same in decerebrate geese during electrically induced walking as in intact birds. Although the decerebrate geese were only made to perform at very low work rates in the present study, our data suggest that, over this low work range, the ventilatory responses to electrically induced locomotion in decerebrate geese are very similar to those seen in intact birds.

$\dot{V}_E$, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ increased significantly in response to walking in both the intact and decerebrate geese, and although the increases were significantly greater in the intact animals, the slopes of the relationships between $\dot{V}_E$ and $\dot{V}_{O_2}$ and $\dot{V}_E$ and $\dot{V}_{CO_2}$ were similar for both groups (Fig. 3). Thus, for a given increase in work rate, the decerebrate and intact birds responded with a similar increase in $\dot{V}_E$. It would have been useful to have extended this comparison to higher work rates. Although higher work rates could be produced with greater stimulation intensities, high-intensity stimulation frequently led to a decrease in the duration of each run or a decrease in fs as stimulation progressed, probably because of increased electrolytic damage to the surrounding tissue. That this decline in activity was not due to fatigue was indicated by the observation that stimulation at new brainstem sites could still elicit high levels of activity. For the purpose of our experiments, it was more important to produce longer periods of steady locomotion repeatedly at low work rates and thus the decerebrate birds were not run at the higher work rates.

In addition, although the sample sizes for the blood gas and pHa values for the intact geese limit our ability to make a reliable comparison between intact and decerebrate geese, it appears from the literature that the blood gas and pHa responses of intact chickens to exercise (Brackenbury & Gleeson, 1983) are similar to those of decerebrate geese. Although Pa_{CO_2} has frequently been observed to decrease and Pa_{O_2} to increase during exercise (intact geese, Table 1; Kiley *et al.*

1979), when exercise is performed isothermically, changes in blood gases and pHa are greatly reduced or absent (decerebrate geese, Table 1; Brackenbury & Gleeson, 1983). Therefore, the larger changes in arterial blood gas tensions observed in intact birds in other studies are probably due to the greater rise in T_b they demonstrate relative to the decerebrate birds described here. It seems likely, therefore, that overall ventilatory control during locomotion is not greatly affected by decerebration.

The effects of decerebration on the control of respiratory pattern were less clear. At rest, breathing patterns were very similar in both groups of birds. With the onset of walking, however, increases in V_T and f_V were both responsible for the steady-state increases in $\dot{V}_E$ in the decerebrate birds (25·2 and 14·6 %, respectively), whereas increases in f_V were solely responsible for the increase in $\dot{V}_E$ in the intact birds (Table 1, Fig. 2). It has been documented that both V_T and f_V increase to produce the increase in $\dot{V}_E$ during isothermic exercise in birds (Brackenbury *et al.* 1982; Brackenbury & Gleeson, 1983). However, when T_b is not controlled and rises during locomotion, breathing frequency continues to rise while V_T decreases to control levels or below (Brackenbury *et al.* 1982). In the present study, the decerebrate geese showed a maximum increase in T_b of only 0·2 °C during walking, while the one intact animal in which T_b was monitored underwent a 0·4 °C increase. Thus, the differences in breathing pattern responses of the two groups to exercise may have been the direct consequence of different thermoregulatory influences on respiration. Similarly, apart from developing a hypocapnia, the ventilatory responses of decerebrate cats to electrically or chemically induced locomotion are very similar to those of normal intact animals (Eldridge *et al.* 1985). In addition, it now appears that exercise hyperpnea is not isocapnic in most species. Dogs, ponies, rats, goats, lizards (Dempsey *et al.* 1985) and birds (Kiley *et al.* 1979) develop hyperventilation and hypocapnia during exercise.

Effects of work rate and decerebration on entrainment

Despite the attention given to the control of ventilation during locomotion in birds (Kiley *et al.* 1979, 1982; Brackenbury *et al.* 1981a,b, 1982; Brackenbury & Gleeson, 1983; Brackenbury, 1986), no one has directly examined the relationship between ventilatory pattern and hindlimb locomotor rhythms in these animals. Substantial work has been performed on humans in this regard and, although it now appears that under appropriate conditions there can be a high degree of coupling between locomotor and respiratory systems during bipedal running (Bramble, 1983; Paterson *et al.* 1987), the mechanisms responsible for this entrainment remain unclear. In particular, based on existing data, it is difficult to isolate the effects of increased limb movement during walking/running from the separate effects of a secondarily produced increase in work rate (Bechbache & Duffin, 1977).

When limb movement rates were controlled in intact and decerebrate geese, entrainment rates were virtually identical in spite of threefold differences in

increases in $\dot{V}_{O_2}$ (Table 1). Associated with the threefold difference in $\dot{V}_{O_2}$ increase between the two groups during walking, the breathing frequencies of the intact group were also three times greater than those of decerebrate birds. In turn, since both groups walked with similar step frequencies, the number of steps taken per breath by the intact geese was approximately one-third of the number taken per breath by the decerebrate birds. Thus, although the number of steps taken per breath is related to metabolic rate, it appears that the degree to which locomotor and respiratory patterns are entrained is completely independent of metabolic work rate during bipedal locomotion in geese. Increased limb movement rate may, therefore, prove to be a major factor associated with increased entrainment in these types of experiments. Afferent information from exercising limbs has been shown to play a role in the entrainment of respiration rate with limb movements (Iscoe & Polosa, 1976; Kawahara et al. 1988), as have central nervous system feedforward effects (Viala et al. 1987).

Although the results of this study do not help elucidate the relative contributions of afferent versus central efferent interactions in developing entrainment, they do serve to isolate the level at which these interactions take place. Some investigators have suggested that locomotor–respiratory coupling in man is under complete, or at least partial, telencephalic control (Yonge & Petersen, 1983). This suggestion is supported by the tight coupling of locomotor and breathing rhythms in trained athletes versus the complete lack of entrainment in sedentary subjects (Bramble, 1983). The increase in entrainment associated with audible pacing cues in humans during cycle exercise (Bechbache & Duffin, 1977; Yonge & Petersen, 1983) also supports this hypothesis. Our data, however, suggest that, although coupling ratios in intact and decerebrate birds are different, removal of the telencephalon and thalamus has no effect on the degree of entrainment between locomotor and respiratory patterns in geese (Table 1).

Humans running on treadmills show rates of entrainment (45 %; Paterson et al. 1987) slightly greater than those seen in geese. In addition, during free running in man, entrainment rates as high as 80 % have been observed (Paterson et al. 1987), suggesting that a moving treadmill forces an unnatural locomotor rhythm, thereby decreasing the level of entrainment (Paterson et al. 1987). Geese may also show higher rates of entrainment during free running. It is also possible that geese never demonstrate entrainment much more than 30 % of the time and that the extra 15–50 % seen in trained humans during free running is learned. If this is the case, it is difficult to envisage the physiological significance of these relatively low coupling rates in geese. However, investigations are currently under way in intact geese to determine if this level of coupling increases under different conditions.

Conclusion

It has been argued that the entrainment of locomotor and ventilatory patterns during human bipedal locomotion may be strongly affected by telencephalic factors (Bechbache & Duffin, 1975; Yonge & Petersen, 1983). The findings of

the present study using another biped, the Canada goose, however, suggest that the telencephalon is not essential for the low degree of coupling seen between the locomotor and respiratory patterns during treadmill walking in this species. Locomotor–respiratory interactions within the brainstem and/or spinal cord are sufficient to produce significant coupling of locomotor and breathing rhythms during bipedal locomotion. In addition, having controlled for the possible effects of limb movement rate on entrainment, this study suggests that, although the number of steps taken per breath is related to work rate, the degree of locomotor–respiratory synchronization is completely independent of metabolic work rate.

References

ADAMS, R. D. (1980). Pain. In *Harrison's Principles of Internal Medicine*, ninth edition (ed. K. J. Isselbacher, R. D. Adams, E. Braunwald, R. G. Petersdorf & J. D. Wilson), pp. 13–20. New York: McGraw-Hill.

BAUDINETTE, R. V., GANNON, B. J., RUNCIMAN, W. B., WELLS, S. & LOVE, J. B. (1987). Do cardiorespiratory frequencies show entrainment with hopping in the tammar wallaby? *J. exp. Biol.* **129**, 251–263.

BECHBACHE, R. R. & DUFFIN, J. (1977). The entrainment of breathing frequency by exercise rhythm. *J. Physiol. Lond.* **272**, 553–561.

BRACKENBURY, J. H. (1986). Blood gases and respiratory pattern in exercising fowl: comparison in normoxic and hypoxic conditions. *J. exp. Biol.* **126**, 423–431.

BRACKENBURY, J. H., AVERY, P. & GLEESON, M. (1981*a*). Respiration in exercising fowl. I. Oxygen consumption, respiration rate and respired gases. *J. exp. Biol.* **93**, 317–325.

BRACKENBURY, J. H. & GLEESON, M. (1983). Effects of P_{CO_2} on respiratory pattern during thermal and exercise hyperventilation in domestic fowl. *Respir. Physiol.* **54**, 109–119.

BRACKENBURY, J. H., GLEESON, M. & AVERY, P. (1981*b*). Effects of sustained running exercise on lung air-sac gas composition and respiratory pattern in domestic fowl. *Comp. Biochem. Physiol.* **69**A, 449–453.

BRACKENBURY, J. H., GLEESON, M. & AVERY, P. (1982). Respiration in exercising fowl. III. Ventilation. *J. exp. Biol.* **96**, 315–334.

BRAMBLE, D. M. (1983). Respiratory patterns and control during unrestrained human running. In *Modelling and Control of Breathing* (ed. B. J. Whipp & D. M. Wiberg), pp. 213–220. New York: Elsevier Biomedical.

BRAMBLE, D. M. & CARRIER, D. R. (1983). Running and breathing in mammals. *Science* **219**, 251–256.

Compendium of Pharmaceuticals and Specialties, XVII edition, (1982). (ed. C. M. E. Krogh, C. B. Schneider, C. Shaugnessy & L. Welbanks), p. 242. Toronto: Southam Murray.

DEMPSEY, J. A., VIDRUK, E. H. & MITCHELL, G. S. (1985). Pulmonary control systems in exercise: update. *Fedn. Proc. Fedn Am. Socs exp. Biol.* **44**, 2260–2270.

ELDRIDGE, F. E., MILLHORN, D. E., KILEY, J. P. & WALDROP, T. G. (1985). Stimulation by

central command of locomotion, respiration and circulation during exercise. *Respir. Physiol.* **59**, 313–337.

FEDDE, M. R., BURGER, P. E., GEISER, J., GRATZ, R. K., ESTAVILLO, J. A. & SCHEID, P. (1986). Effects of altering dead space volume on respiratory and air sac gases in geese. *Respir. Physiol.* **66**, 109–122.

FELDMAN, J. L., SMITH, J. C., McCRIMMON, D. R., ELLENBERGER, H. H. & SPECK, D. F. (1988). Generation of respiratory pattern in mammals. In *Neural Control of Rhythmic Movements in Vertebrates* (ed. A. H. Cohen, S. Rossignol & S. Grillner), pp. 73–100. New York: John Wiley & Sons.

GRILLNER, S. (1975). Locomotion in vertebrates: central mechanisms and reflex interaction. *Physiol. Rev.* **55**, 247–304.

HART, J. S. & ROY, O. Z. (1966). Respiratory and cardiac responses to flight in pigeons. *Physiol. Zool.* **39**, 291–306.

ISCOE, S. & POLOSA, C. (1976). Synchronization of respiratory frequency by somatic afferent stimulation. *J. appl. Physiol.* **40**, 138–148.

JASINSKAS, C. L., WILSON, A. B. & HOARE, J. (1980). Entrainment of breathing rate to movement frequency during work at two intensities. *Respir. Physiol.* **42**, 199–209.

KAWAHARA, K., KUMAGAI, S., NAKAZONO, Y. & MIYAMOTO, Y. (1988). Analysis of entrainment of respiratory rhythm by somatic afferent stimulation in cats using phase response curves. *Biol. Cybernetics* **58**, 235–242.

KAY, J. D. S., PETERSEN, E. S. & VEJBY-CHRISTENSEN, H. (1975). Breathing in man during steady-state exercise on the bicycle at two pedalling frequencies, and during treadmill walking. *J. Physiol., Lond.* **251**, 645–656.

KILEY, J. P., KUHLMANN, W. D. & FEDDE, M. R. (1979). Respiratory and cardiovascular responses to exercise in the duck. *J. appl. Physiol.* **47**, 827–833.

KILEY, J. P., KUHLMANN, W. D. & FEDDE, M. R. (1982). Ventilatory and blood gas adjustments in exercising isothermic ducks. *J. comp. Physiol.* **147**, 107–112.

KOHL, J., KOLLER, E. A. & JAGER, M. (1981). Relation between pedalling and breathing rhythm. *Eur. J. appl. Physiol.* **47**, 223–237.

LOESER, J. D. & BLACK, R. G. (1975). A taxonomy of pain. *Pain* **1**, 81–84.

LUMSDEN, T. (1923). Observations on the respiratory centres in the cat. *J. Physiol., Lond.* **59**, 153–160.

McCLELLAN, A. D. (1986). Command systems for initiating locomotion in fish and amphibians: parallels to initiation systems in mammals. In *Neural Control of Vertebrate Locomotion* (ed. S. Grillner, R. M. Herman, P. S. G. Stein, H. Forsberg & D. G. Stuart), pp. 3–20. London: Macmillan Press.

NOMOTO, S., RAUTENBERG, W. & IRIKI, M. (1983). Temperature regulation during exercise in the Japanese quail (*Coturnix coturnix japonica*). *J. comp. Physiol.* **149**, 519–525.

PATERSON, D. J., WOOD, G. A., MARSHALL, R. A., MORTON, A. R. & HARRISON, A. B. C. (1987). Entrainment of respiratory frequency to exercise rhythm during hypoxia. *J. appl. Physiol.* **62**, 1767–1771.

PATERSON, D. J., WOOD, G. A., MORTON, A. R. & HENSTRIDGE, J. D. (1986). The entrainment of ventilation frequency to exercise rhythm. *Eur. J. Physiol. occup. Physiol.* **55**, 530–537.

PAVLIDIS, T. (1973). *Biological Oscillations: Their Mathematical Analysis.* New York: Academic Press.

SCHMIDT-NIELSEN, K. (1979). *Animal Physiology. Adaptation and Environment*, 2nd edn. pp. 162–163. New York: Cambridge University Press.

SHERRINGTON, C. S. (1910). Flexion-reflex of the limb, crossed extension reflex, and reflex stepping and standing. *J. Physiol., Lond.* **40**, 28–121.

SHERRINGTON, C. S. (1915). Simple apparatus for obtaining a decerebrate preparation of the cat. *J. Physiol. Lond.* **49**, 1ii–1iv.

SHOLOMENKO, G. N. & STEEVES, J. D. (1987). Effects of spinal cord lesions on hind limb locomotion in birds. *Expl Neurol.* **95**, 403–418.

STEEVES, J. D., SHOLOMENKO, G. N. & WEBSTER, D. M. S. (1987). Stimulation of the pontomedullary reticular formation initiates locomotion in decerebrate birds. *Brain Res.* **401**, 205–212.

VIALA, D., PERSEGOL, L. & PALISSES, R. (1987). Relationship between phrenic and extensor activities during fictive locomotion. *Neurosci. Lett.* **74**, 49–52.

WALL, P. D. (1975). Editorial. *Pain* **1**, 1–2.

YONGE, R. P. & PETERSEN, E. S. (1983). Entrainment of breathing in rhythmic exercise. In *Modelling and Control of Breathing* (ed. B. J. Whipp & D. M. Wiberg), pp. 197–204. New York: Elsevier Biomedical.