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Overground locomotion in intact rats: interlimb coordination, support patterns and support phases duration

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Abstract. The interlimb coordination during overground locomotion was analysed in intact rats, using the method of contact electrodes (Górska et al. 1998). It was found that in animals moving with a speed ranging from 10 to 78 cm/s (step cycles 685 to 215 ms, respectively) the interlimb coordination was characterized by homologous phase shifts close to 0.5 and much shorter diagonal than lateral phase shifts. These features corresponded to symmetrical gait with diagonal sequence and diagonal couplets (Hildebrandt 1976). Shortening the step cycle changed the gait from a walking trot (duty factor >0.5) into a running trot (duty factor <0.5). Correspondingly, the support patterns in the four-legged step cycles, i.e., the sequence of phases of support on various limbs changed: the support on diagonal limbs persisted but the three-limb support was replaced by one-limb support and the support on homolateral limbs by phases of flight. For each phase of support the relationship between its absolute and relative durations and the step cycle duration is being described. The paper explains the variability of support patterns described in the literature. The picture of locomotion obtained in intact rats will be used as a template for studying locomotor control deficits after CNS lesions.

Key words: rat, overground locomotion, interlimb coordination, support patterns

INTRODUCTION

In our previous paper on the overground locomotion in intact rats using the method of contact electrode recordings (Górska et al. 1998), the basic indices of gait were analyzed and compared, whenever possible, with the data in the literature obtained mainly with video or cinematographic records. It was found that the method of contact electrodes gives reliable and reproducible results and may be used in further studies on rat locomotion.

The aim of the present paper was to describe the interlimb coordination in overground locomotion in rats. This problem has only been a little dealt with in the previous literature and the published data have not provided sufficient information about all the variables describing the gait and their relationships with the step cycle duration (Cohen and Gans 1975, Hruska et al. 1979, Gruner et al. 1980, Molinari and Petrosini 1993). In addition, because locomotion in animals can also be described in terms of support patterns, i.e., the sequence of configurations of supports on various limbs in different epochs of the four-legged step cycle (Howell 1944, Muybridge 1957, Hildebrandt 1965, 1966, 1976, Roberts 1967, Gray 1968 and others), we have performed such an analysis for better interpretation of the obtained results. For each step the support formula has been defined and for each support phase the conditions which have to be fulfilled for its appearance and changes in its absolute and relative durations as a function of the step cycle duration have been described. It will be shown that such an analysis has made it possible to establish the relations between the step cycle duration, interlimb coordination and the support pattern formulae. The obtained results explain the diversity of support patterns described in the literature in rats (Cohen and Gans 1975, Hruska et al. 1979, Gruner et al. 1980, Molinari and Petrosini 1993) and can be easily adapted for symmetrical gaits in other tetrapods. They will serve as a template for studying changes in fore-hindlimb coordination in rats after partial spinal lesions. Part of the results have been published in abstract form elsewhere (Zmysłowski et al. 1995).

METHODS

The animals and the recording procedures were the same as in our previous paper (Górska et al. 1998). Briefly, seven adult hooded rats of either sex were trained to move at a relatively constant speed along a runway (2 m long, 0.12 m wide) covered with an aluminium sheet connected to a 100 mV DC source. The animals had contact electrodes on each paw to record the stance phases. A photocell system was used to measure the locomotor speed. In each animal at least 100 step cycles, performed in about 20 passes (sequence of 5-12 steps per pass performed with a velocity differing by not more than 10%) were collected for analysis. The time measurement errors were estimated to be 5 ms, while the speed measurements errors between two adjacent photocells (placed 25 cm apart) were 0.01 m/s.

The analysis of interlimb coordination included: (1) the homologous, homolateral and diagonal time and phase (ratio of time to step cycle duration) shifts; (2) the support formula in the four-legged step cycle, i.e., the sequence of configurations of support on various limbs during the step cycle; (3) the absolute and relative durations of various phases of support in the four-limb step cycle; (4) the relationships between these variables and the step cycle duration. In addition, the duty factors, i.e., the relative duration of the stance phases (ratio of stance phase duration to the step cycle duration) were counted for each limb in each step.

All the relationships were evaluated by regression (least square method) and correlation analysis. In all cases $P<0.01$ was considered significant.

RESULTS

Basic gait indices

The basic gait indices such as the locomotor speed, the stance, swing and step cycle durations and the relationships between these variables had been described in detail in our previous paper (Górska et al. 1998). Briefly, the mean locomotor speed in individual animals ranged between 25 ± 6 (rat No. 6) and 45 ± 7 cm/s (rat No. 2) with the minimal and maximal speed of 10 and 78 cm/s, respectively. This corresponded to the mean step cycle duration ranging between 304 ± 85 (No. 2) and 512 ± 102 ms (No. 6) (range in individual steps 215-685 ms), the mean stance phase duration ranging between 135 ± 36 and 337 ± 92 ms (range in individual steps 80-405 ms) and mean swing phase duration ranging between 146 ± 16 and 243 ± 24 ms (range in individual steps 105-310 ms).

The decrease in the step cycle, stance and swing phases durations with increase of the locomotor speed could be fitted best by using a power function $y=ax^b$.

These relationships were similar for the step cycle and stance phases durations (log *a*: 7.60 to 8.51 and 7.57 to 9.08; *b*: -0.46 to -0.71 and -0.58 to -1.04; *r*: -0.67 to -0.92 and -0.60 to -0.90, respectively), while much weaker for the swing phase (log *a:* ns to 7.63; *b*: -0.13 to -0.57; *r*: ns to -0.71). On the other hand, the relationships between the stance and swing durations and the step cycle duration were linear, with the slopes of the stance phase being much steeper than for the swing phase (slopes of regression lines for the stance 0.63 to 0.88, for the swing 0.12 to 0.37).

The interlimb differences were limited to the stance and swing phases and were not correlated with the step cycle duration. The interanimal variability in the step cycle durations did not exceed 100 ms, i.e., was similar to that described for cats (Halbertsma 1983, Vilensky and Patrick 1984, Górska et al. 1993a).

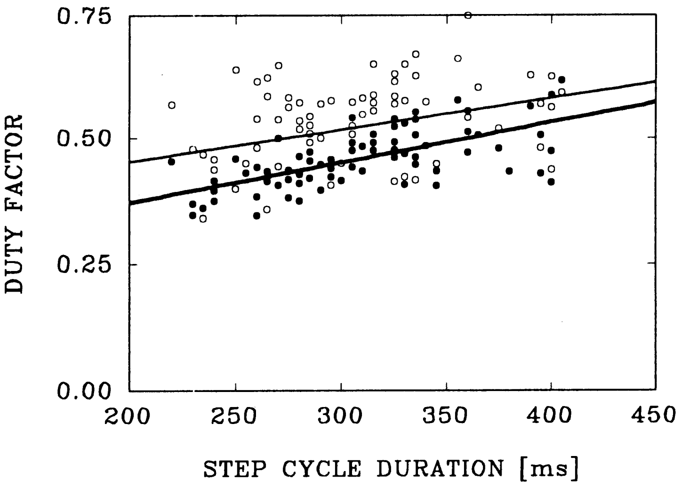

Fig. 1. Relationships between the duty factor and the step cycle duration. Thick line and filled symbols, left forelimb (LF); thin line and empty symbols, right hindlimb (RH) (rat No. 2).

Duty factor

The mean duty factor, i.e., the ratio of the stance phase to the step cycle duration, differed in various limbs and individual rats in a similar way as the stance phase duration. The mean duty factor in the forelimbs ranged between 0.42 ± 0.07 (No. 2) and 0.68 ± 0.04 (No. 6), and between 0.42 ± 0.04 (No. 1) and 0.61 ± 0.05 (No. 6) in the hindlimbs. The minimal and maximal values of the duty factor were 0.29 and 0.79, respectively. In some rats (Nos. 2, 4 and 5) the mean duty factor in both hindlimbs was longer, while in the remaining animals shorter, than in the forelimbs. In all the rats (except No. 6 limbs RH, LH and RF) the duty factor in each limb was correlated with the step cycle duration, (*a* ranged between 0.34 and 0.83 s^{-1}, *r* between 0.27 and 0.86) (Fig.1).

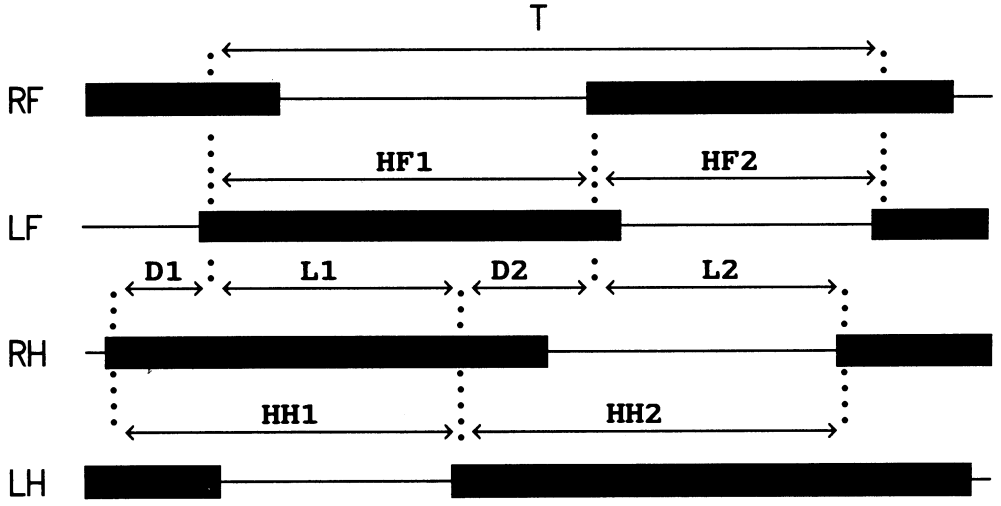

Fig. 2. Footfall diagram in the four-limb step cycle. Thick lines denote stance phases, thin lines swing phases of each limb. RF, LF, RH and LH denote the right and left forelimb and right and left hindlimb, respectively. T, step cycle of the left forelimb. HF and HH, homologous shifts (difference between the onsets of stance phases in the pair of fore- (HF1 and HF2) and hindlimbs (HH1 and HH2)); D1 and D2, diagonal shifts (difference between the onsets of stance phases in one pair of diagonal limbs); L1 and L2, homolateral shifts (difference between the onsets of stance phases in one pair of homolateral limbs).

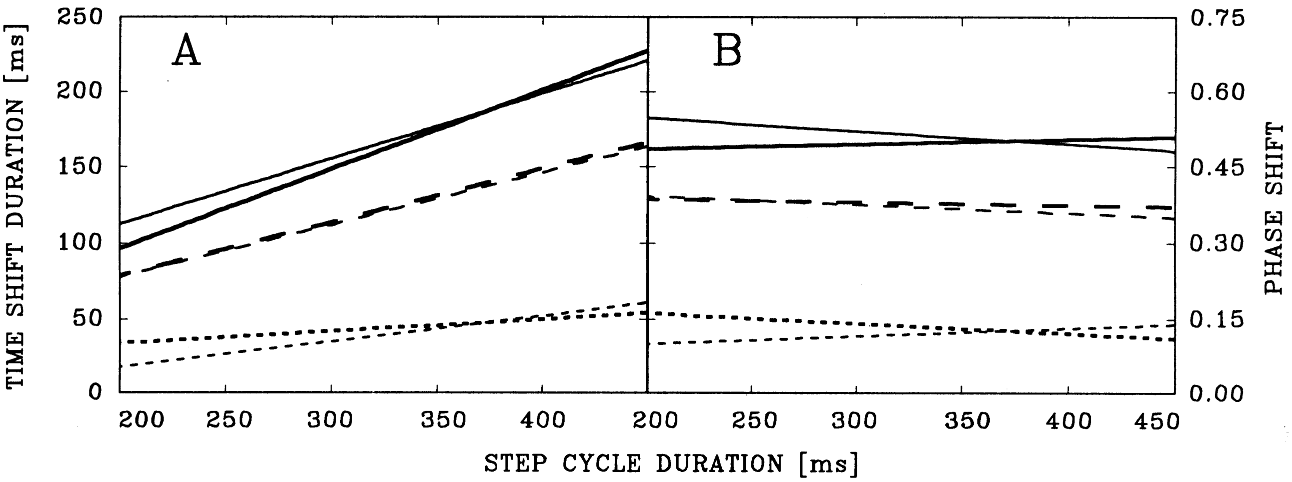

Fig. 3. Relationships between the absolute (A) and relative (B) durations of homologous (HF1, thick solid line; HH1, thin solid line), homolateral (L1, thick broken line; L2, thin broken line) and diagonal (D1, thick dotted line; D2, thin dotted line) shifts and the step cycle duration. For the explanation of shifts see Fig. 2.

Interlimb coordination

HOMOLOGOUS TIME AND PHASE SHIFT

The mean homologous time shift, i.e., the time between the onsets of the stance phases in the pair of homologous limbs (see Fig. 2) varied from 149 ± 26 to 244 ± 50 ms and from 156 ± 26 to 261 ± 57 ms in the fore- and hindlimbs, respectively. This corresponded to the mean phase shift (ratio of time shift to the step cycle duration) ranging between 0.42 ± 0.04 and 0.58 ± 0.05 in the forelimbs and between 0.44 ± 0.05 and 0.56 ± 0.06 in the hindlimbs (Table I). The homologous time shifts were positively correlated with the step cycle duration, i.e. increased with the prolongation of the step cycle duration: *a* ranged between 0.45 and 0.60, *r* between 0.81 and 0.93 (Fig. 3A). On the other hand, the homologous phase shifts were not correlated with the step cycle duration (Fig. 3B). This shows that the animals used a symmetrical gait irrespective of the speed.

In the majority of rats neither the homologous time nor the phase shifts in both fore- and hindlimbs were

TABLE I

The means ± standard deviation of the homologous, diagonal and homolateral phase shifts. For explanation of shifts and their denotations cf. Fig. 2

	Homologous				Diagonal		Homolateral	
Rat	HF1 LF→RF	HF2 RF→LF	HH1 RH→LH	HH2 LH→RH	D1 RH→LF	D2 LH→RF	L1 LF→LH	L2 RF→RH
No 1	0.48 ± 0.05	0.52 ± 0.05	0.52 ± 0.04	0.48 ± 0.04	0.08 ± 0.08	0.04 ± 0.05	0.44 ± 0.03	0.44 ± 0.05
No 2	0.49 ± 0.06	0.51 ± 0.04	0.52 ± 0.04	0.48 ± 0.04	0.14 ± 0.06	0.11 ± 0.05	0.38 ± 0.05	0.37 ± 0.06
No 3	0.54 ± 0.07	0.46 ± 0.06	0.50 ± 0.04	0.50 ± 0.05	0.09 ± 0.04	0.13 ± 0.06	0.41 ± 0.05	0.37 ± 0.08
No 4	0.53 ± 0.06	0.47 ± 0.05	0.49 ± 0.04	0.51 ± 0.04	0.00 ± 0.05	0.04 ± 0.05	0.49 ± 0.05	0.47 ± 0.06
No 5	0.42 ± 0.04	0.58 ± 0.05	0.56 ± 0.06	0.44 ± 0.05	0.21 ± 0.06	0.07 ± 0.03	0.35 ± 0.04	0.37 ± 0.05
No 6	0.47 ± 0.04	0.53 ± 0.06	0.50 ± 0.05	0.50 ± 0.06	0.13 ± 0.04	0.10 ± 0.04	0.37 ± 0.05	0.40 ± 0.05
No 7	0.42 ± 0.04	0.58 ± 0.05	0.50 ± 0.03	0.50 ± 0.04	0.13 ± 0.04	0.05 ± 0.04	0.37 ± 0.05	0.45 ± 0.05

equal. The mean differences in both homologous phase shifts in the forelimbs (HF1 and HF2) and in the hind-limbs (HH1 and HH2) (Fig. 2) were significant in most rats and ranged from -0.15 ± 0.06 to 0.08 ± 0.08 and from -0.02 ± 0.01 to 0.11 ± 0.09, respectively.

DIAGONAL AND LATERAL TIME AND PHASE SHIFTS

In all animals, the time elapsing between the stance onsets in the diagonal limbs (D1 and D2 in Fig. 2) i.e., the diagonal time shift, was much shorter than that between the homolateral limbs (L1 and L2 in Fig. 2) i.e., the lateral time shift. The respective mean values ranged between 1 ± 23 and 81 ± 20 ms for the diagonal and between 112 ± 23 and 218 ± 35 for the lateral time shifts (Table I). This corresponded to mean phase shifts (time shift divided by step duration): diagonal ranging between 0.0 ± 0.05 and 0.21 ± 0.06 and lateral ranging between 0.35 ± 0.04 and 0.49 ± 0.05 (Table I).

In the vast majority of animals, the diagonal and lateral time shifts were correlated (Fig. 3A) with the step cycle duration (diagonal, *a:* 0.08 to 0.22, *r:* 0.17 to 0.62; lateral, *a*: 0.28 to 0.48; *r:* 0.40 to 0.86). On the other hand, the diagonal and lateral phase shifts were not correlated, or only weakly, (Fig. 3B) with the step cycle duration: usually only one or at the most two out of the four phase shifts in each animal showed some weak correlation with the step cycle duration ($r = 0.21$ to 0.46). In all the animals the diagonal phase shifts were negatively correlated with the lateral ones (*a*: -0.30 to -0.87, *r*: -0.24 to -0.70).

The two diagonal and the two lateral shifts differed from each other. The mean differences between the two diagonal time shifts in individual rats ranged from -24 ± 28 to 54 ± 19 ms, while between the two lateral time shifts from -31 ± 36 to 18 ± 42 ms. The differences between the two diagonal and the two lateral time shifts in single steps were nonsignificant or weakly correlated with the step cycle duration ($r<0.28$).

PATTERN OF SUPPORT AND DURATION OF SUPPORT PHASES

The representative examples of steps performed by rats (Nos. 2 and 6) at different speeds are shown in Fig. 4. Solid lines show the relative duration of the stance phase in each limb. The step cycles are of different duration. Below each graph phases of support on different limbs in various epochs of the four-legged step cycle are schematically represented.

In the vast majority of steps the animals used the order of footfalls: left forelimb, left hindlimb, right forelimb and right hindlimb (LF,LH,RF,RH) in which a footfall of a hindlimb was preceded by a footfall of the homolateral forelimb and followed, after a short interval (diagonal phase shift), by a footfall of the diagonal forelimb (Fig. 4). Hildebrandt (1966, 1976) called this kind of gait diagonal sequence, diagonal couplets, because the footfall of a given hindfoot was followed by a footfall of a forefoot on the opposite side (diagonal sequence) and both these footfalls were related in time as a pair (diagonal couplets). This last feature is characteristic for trotting, in which diagonally opposite legs move more or less together.

Depending on the percent of step time each foot is on the ground, i.e., the duty factor, Hildebrandt (1966, 1976) distinguished the walking trot in which the foot is on the ground more than half of the cycle and running trot in which the foot is on the ground less than half of the cycle. Furhermore, he divided the walking trot into slow (duty factor (DF) 0.70-0.80), moderate (DF 0.60-0.70), and fast (DF 0.50-0.60), and similarly he did for the running trot (DFs: slow, 0.40-0.50, moderate, 0.30-0.40, fast, 0.20-0.30). Since the duty factor in our rats ranged from 0.79 to 0.29, the gait could be classified as ranging from a slow walking trot to a moderate running trot. Examples of step cycles performed during moderate (A) and fast (B,C) walking trot and slow (D) and moderate (E) running trot are shown in Fig. 4. In all these steps the diagonal phase shifts ranged between 0.02 and 0.16, while the lateral shifts between 0.34 and 0.47 (Table II).

The analysis of the support phases, i.e., the configuration of limbs supporting the body in various epochs of the four-limb step cycle (cf. lower row in each gait diagram in Fig. 4) showed that, with the decrease in step cycle duration, the support formula changed from that typical for walking, in which phases of support on three limbs alternated with supports on two limbs, diagonal and lateral, (Fig. 4A) (formula 8 according to Hildebrandt, 1976) into a formula typical for running, in which the supports on both diagonal limbs were separated by supports on one limb and phases of flight (Fig. 4D and E) (formula 13 and 14, Hildebrandt, 1976). The main factor contributing to the change in the support formula was the decrease of the duty factor in each limb, while other variables like the homologous, homolateral and diagonal phase shifts remained relatively stable (Table II). Below, we describe the conditions under which different phases of support appeared and the relationships between their absolute and relative durations and the step cycle duration.

Support on three limbs

The support on three limbs appeared only in steps with the duty factor longer than the homologous phase shift, i.e., in walking. They were the result of an overlapping of the onset of the stance phase in one limb with the offset of stance phase in the second homologous limb and, at the same time, with the stance phase of one limb of the other girdle, set down previously (Fig. 4A-C).

In long steps with a relatively large duty factor (as e.g. on Fig. 4A), one step involved four phases of three-legged support, two on both fore- and one hindlimb and two on both hind- and one forelimb. Each of these phases was equal to the time elapsing between the onset and offset of stances in the respective pair of homologous limbs. They equalled, therefore, the stance phase duration of a given limb minus the homologous shift between this limb and the other one of the pair (e.g. Fig. 4A and Table II, duration of support on LF+RF+LH at the beginning of the second half of the cycle: DFLF - HF1 = 0.25).

If the duty factor of a given limb was equal to the homologous phase shift, the support on three limbs was absent. On the other hand, in steps in which the duty factor was smaller than the homologous phase shift, like in running, the phases of three-legged support were replaced by the support on one leg (see below).

In the present experiments all four three-legged support phases appeared in 100% of steps only in rat No. 6, which was the slowest one (mean speed 25 ± 6 cm/s, mean step cycle 502 ± 87ms, cf. Table I in Górska et

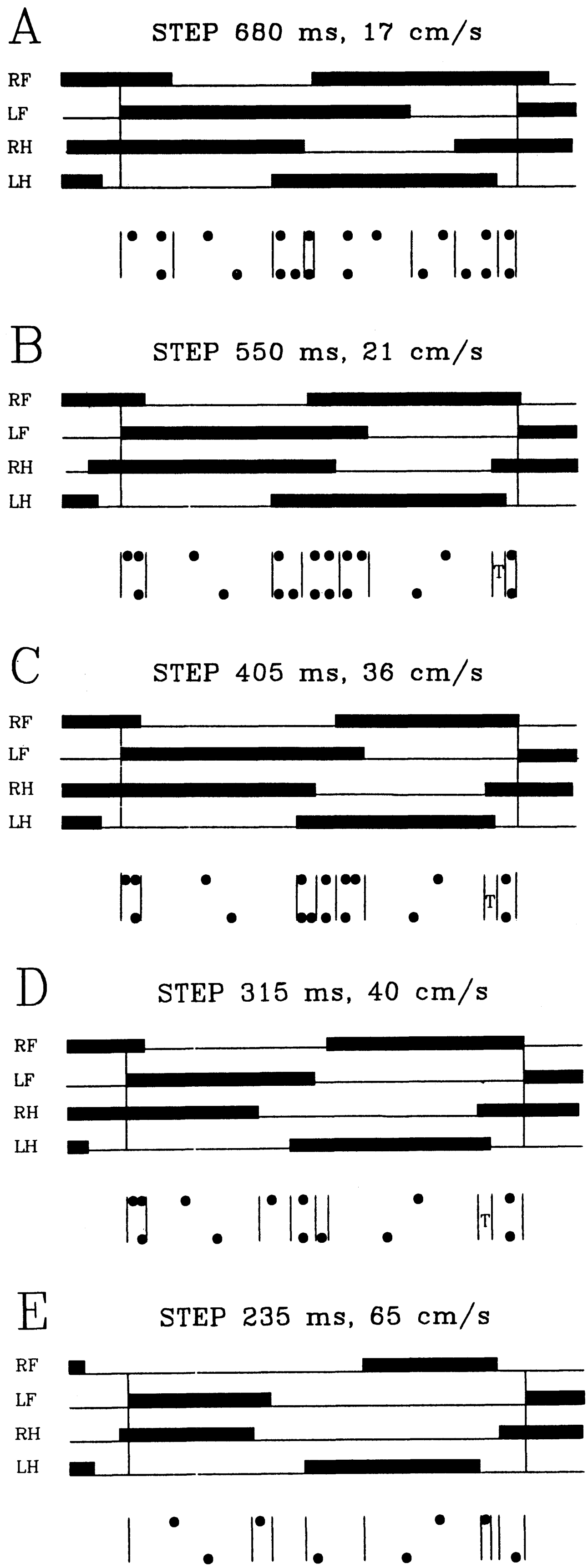

Fig. 4. Representative examples of footfall diagrams and support patterns in steps of different durations performed by rats. In each step thick lines denote the stance phase, thin lines the swing phase of each limb. The step cycle duration (of LF limb) and the corresponding locomotor speed are shown above each diagram. Dots below each diagram symbolize limbs supporting the body in successive epochs of the step cycle (support phases) and their relative durations. In B,C and D phases of support on three limbs shorter than 15 ms are denoted by T. A and C, typical pattern of support during walking (A, moderate, C, fast); B, some its modification with the four-limb support in the middle of step; D, pattern of support during slow run; E, pattern of support during moderate run. For the values of duty factors, homologous, lateral and diagonal phase shifts see Table II. Abbreviations as in Fig. 2. (A-B rat No. 6, C-E rat No. 2).

TABLE II

Duty factors, homologous, diagonal, and lateral phase shifts in steps shown in Fig. 4

Respective step shown in Figure 4		A	B	C	D	E
	RF	0.60	0.55	0.47	0.49	0.32
Duty	LF	0.73	0.63	0.62	0.48	0.36
Factor	RH	0.59	0.63	0.64	0.49	0.34
	LH	0.57	0.59	0.51	0.51	0.45
	HF1: LF→RF	0.48	0.47	0.54	0.51	0.60
Holomogous	HF2: RF→LF	0.52	0.53	0.46	0.49	0.40
Phase Shift	HH1: RH→LH	0.51	0.46	0.59	0.57	0.47
	HH2: LH→RH	0.46	0.56	0.48	0.48	0.49
Diagonal Phase	D1: RH→LF	0.13	0.08	0.15	0.16	0.02
Shift	D2: LH→RF	0.10	0.09	0.10	0.10	0.15
Homolateral	L1: LF→LH	0.38	0.38	0.44	0.41	0.45
Phase Shift	L2: RF→RH	0.36	0.47	0.38	0.38	0.34
Kind of gait and		Moderate	Fast	Fast	Slow	Moderate
Duty Factor		walking	Walking	walking	running	running
(Hildebrandt, 1966)		0.60-0.70	0.50-0.60	0.50-0.60	0.40-0.50	0.30-0.40

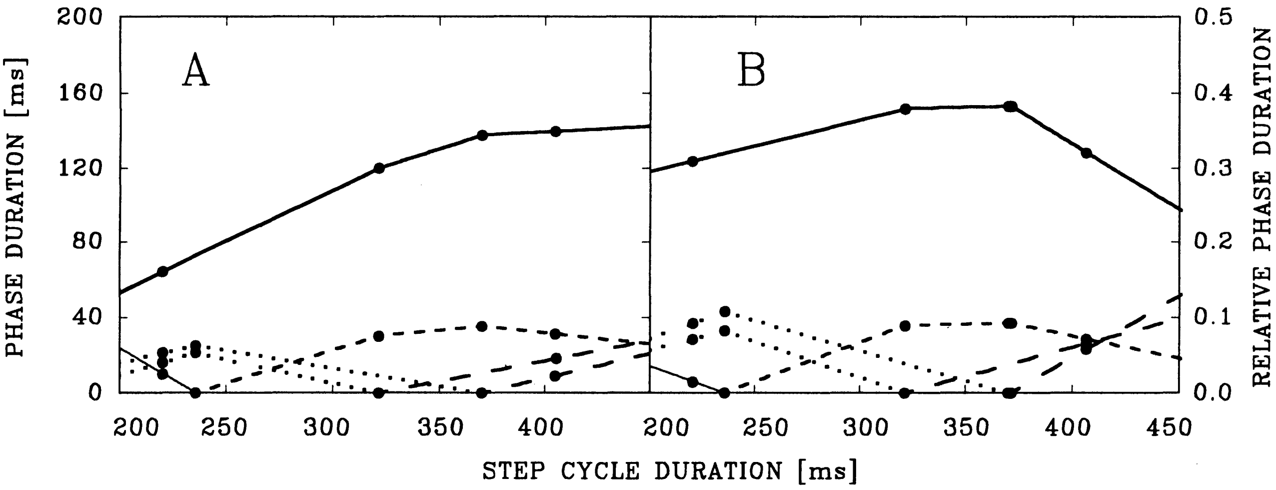

Fig. 5. Relationships between the absolute (A) and relative (B) durations of different support phases and the step cycle duration (rat No. 2). For the sake of clarity only the regression lines for the support phases appearing in the 1st half of the step cycle are shown (cf. Fig. 4). Denotations: solid thick line, support on diagonal limbs; broken line long dashes, support on three limbs (upper line: two hind-one fore, lower line: two fore-one hindlimb); broken line short dashes, support on homolateral limbs; dotted lines, support on one limb (upper line - on forelimb, lower line - on hindlimb); thin solid line, phase of flight. The support on four limbs is not shown because it appeared only occasionally (see text). Note shortening in B, with the increase in step cycle duration, of the relative durations of the diagonal and homolateral supports as a result of the appearance of the three-limb support and the replacement of phases of flight by the homolateral support and of one-limb support by the phases of three-limb support.

al.1998). In the remaining rats, the support on both fore- and one hindlimb appeared in 7 (No. 2) to 90% (No. 3) of steps, while the supports on both hind- and one forelimb in 0 (No. 1) to 100% (No. 5) of steps. The mean absolute duration of the supports on both fore- and one hindlimb ranged from 13 ± 12 to 51 ± 35 ms, while for the supports on both hind- and one forelimb from 5 ± 6 to 69 ± 34 ms. This corresponded to the mean relative duration ranging from 0.03 ± 0.02 to 0.10 ± 0.06 and from 0.01 ± 0.01 to 0.13 ± 0.05 for the first and second kind of support, respectively.

The relationships between the absolute and relative durations of the support on three limbs and the step cycle duration for rat No. 2 are shown in Fig. 5A and B, respectively. For the sake of clarity, only the supports appearing in the first half of the step cycles are shown. As seen from the regression lines, the support on both fore - one hindlimb (lower broken line - long dashes in Fig. 5) began to appear in steps of approximately 370 ms, while the support on both hind - one forelimb (upper broken line - long dashes in Fig. 5) in steps of approximately 320 ms. In the remaining cases the three-limb supports begun to appear in step cycles ranging between 220 ms (No. 2 - 2nd half of the step cycle) and 485 ms (No. 4), and no consistency was observed with regard to which of the possible four three-limb supports appeared first in the step cycles. When present, both the absolute and the relative duration of the three-limb supports increased with the prolongation of the step cycle duration (Fig. 5A and B). The slopes for the increase in their absolute values ranged in individual animals between 0.07 and 0.47, while for their relative values between 0.19 and 1.16 s^{-1}. The coefficients of correlation *r* ranged between 0.22 and 0.82 in the first case, while these values were 0.20 and 0.78 in the latter.

Support on four limbs

In some animals the three-legged supports at the beginning of each half of the step cycle were preceded by a four-limb support, which replaced the support on homolateral limbs in the middle of the step cycle. This happened whenever there was an overlapping of supports on both pairs of homologous limbs (Fig. 4B). The duration of this phase of support was equal to the time elapsing between the onset of forelimb stance and the homolateral hindlimb stance offset (RF and RH in Fig. 4B). It equalled, therefore, the hindlimb stance duration minus its diagonal shift and minus the homologous shift of the forelimb (e.g. Fig. 4B and Table II: DFRH - D1 - HF1 = 0.08). If the hindlimb stance duration minus its diagonal shift was shorter than the homologous shift the support on four limbs did not occur (Fig. 4A and C).

In the present experiments, the phases of support on four limbs were observed only in 4 rats walking relatively slowly (Nos. 3, 4, 6 and 7, mean speed 28 ± 5 to 36 ± 9 cm/s). They began to appear at step cycles ranging between 320 (No. 6) and 560 ms (No. 7). They occurred in 5 (No. 7) to 30% (No. 4) of steps and were usually very short (mean for the absolute duration ranged from 10 ± 5 to 26 ± 18 ms; for the relative duration from 0.02 ± 0.02 to 0.05 ± 0.06). The absolute and relative durations of both phases of four-limb support were not correlated with the step cycle duration.

Support on diagonal limbs

The supports on two diagonal limbs appeared in all the animals in 100% of steps. They were the longest in the step cycle. The mean absolute duration of these supports ranged between 95 ± 25 (No. 6) and 184 ± 34 ms (No. 4), which corresponded to the mean relative durations of 0.19 ± 0.06% to 0.41 ± 0.05% of the step cycle.

In steps, with duty factor shorter than the homologous phase shift and hence with no support on three limbs, like in running, the support on diagonal limbs corresponded to time elapsing between the forelimb stance onset and the respective diagonal hindlimb stance offset (Fig. 4E). Its duration equalled, therefore, the stance duration of the given hindlimb minus the corresponding diagonal shift (Fig. 4E and Table II support on LF+RH: DFRH - D1 = 0.32). In such steps, the absolute duration of the phases of support on diagonal limbs positively correlated with the step cycle duration (Fig. 5A steps up to 320 ms), with slopes of linear regressions *a* ranging between 0.37 and 0.68 and the coefficient of correlation *r* between 0.68 and 0.94. On the other hand, the relative duration of the diagonal support phases were either not correlated (Nos. 1 and 4) or only correlated weakly (a: 0.36 to 0.68 s^{-1}; *r* 0.44 to 0.68) (Fig. 5B).

In steps with supports on three limbs (Fig. 4A-C), the durations of the diagonal support phases were curtailed by the neighbouring phases of support on three limbs. In such steps the duration of the support on diagonal limbs equalled the time elapsing between the forelimb stance offset and the diagonal hindlimb stance onset. This corresponded to the stance duration of the hindlimb minus the diagonal shift and minus the duration of the neighbouring three-limb supports (cf Fig. 4A and Table II: support on RF+LH in the second half of the cycle: DFLH - D2 - (the preceding three-legged support equal to DFLF - HF1) and - (the succeding three-legged support equal to DFLH - HH2) = 0.11). As a result, the absolute duration of the supports on diagonal limbs in such steps was either not or only weakly correlated with the step cycle duration (*a*: 0.07 to 0.20; *r*: 0.15 to 0.55) (Fig. 5A, steps longer than 370 ms). On the other hand, the relative durations of the diagonal supports decreased with the increase in the step cycle duration (*a*: -1.22 to -0.32 s^{-1}; *r*: -0.82 to -0.47) (Fig. 5B), due to simultaneous increase of the supports on the three limbs.

Support on homolateral limbs

The phases of support on homolateral limbs occurred in individual rats in 30 (No. 4) to 95% (No. 7) of steps. When present, they were usually short (range of means: absolute duration 10 ± 10 to 42 ± 23 ms; relative duration 0.03 ± 0.03 to 0.10 ± 0.05) and occurred at the end of each half of the step cycle (Fig. 4A,C,D). They began to appear in individual animals in step cycles ranging between 235 (No. 2) to 430 ms (No. 4). In shorter steps, they were replaced by phases of flight (cf. Fig. 4E). In steps with no preceding three-limb support, the duration of the support on homolateral limbs constituted the time interval elapsing between the hindlimb stance onset and the homolateral forelimb stance offset (Fig. 4D). Its duration equalled, therefore, the stance duration of a given forelimb minus its homolateral shift (e.g. Fig. 4D and Table II support on left homolateral limbs: DFLF - L1 = 0.07). These intervals were positively correlated with the step cycle duration (absolute values: *a*: 0.29 to 0.51; *r*, 0.58 to 0.88; relative values: *a*: 0.37 to 1.02 s^{-1}; *r*: 0.47 to 0.81) (Fig. 5A and B, steps shorter than 320 ms).

In steps with three-limb supports, the duration of the homolateral support was curtailed by the preceding and/or the following support on three limbs (Fig. 4A and C). In such steps the support on homolateral limbs constituted the time interval elapsing between hindlimb stance offset and the homolateral forelimb stance onset in the opposite pair of limbs (i.e., right for the support on left limbs and *vice versa*). They equalled, therefore, the respective forelimb stance duration minus its homolateral shift and minus the neighbouring three-limb supports (e.g. Fig. 4A and Table II support on left limbs first half of the cycle: DFLF - L1 - (DFRH - HH1) - (DFLF - HF1) = 0.02). As a result, the absolute duration of the homolateral supports in such steps were not or weakly negatively correlated with the step cycle duration (*a*: -0.13 to -0.09, *r*: -0.35 to -0.25), while their relative durations decreased with the increase of the step cycle durations (*a*: -0.58 to -0.16 s^{-1}; *r*: -0.62 to -0.15) (Fig. 5A and B, steps longer than 370 ms).

As stressed above, the support on homolateral limbs did not occur if the stance duration of a hindlimb minus its diagonal shift was longer than the homologous shift of the contralateral forelimb. In such steps, the homolateral support was replaced by the four-legged support (e.g. Fig. 4B and Table II: DFRH - D1 = 0.55 HF1 = 0.47). If, on the other hand, the stance duration of a forelimb was shorter than the homolateral shift, the support on homolateral limbs was replaced by a phase of flight (Fig. 4E and Table II: DFLF=0.36 < L1=0.45) (see below).

Support on one limb

Phases of support on one limb (fore or hind) appeared in steps in which the duty factor of a given limb was shorter than the homologous phase shift, i.e., during running. Such supports were present in individual animals at step cycles shorter than 320 (No. 2) to 490 ms (No. 5). They occurred in 0 (No. 6 all limbs, No.5 support on LF and RF) to 100% (No. 1, support on LF) of steps and were relatively short (range of means: absolute 6 ± 6 to 36 ± 16 ms, relative 0.02 ± 0.02 to 0.09 ± 0.04). In steps in which the support on homolateral limbs was absent (Fig. 4E), the duration of the support on one limb was equal to the diagonal shift between the offstets (for the support on one forelimb) or the onsets (for the support on one hindlimb) of the stance phases in the respective limbs (Fig. 4E support on left hindlimb equal D2 = 0.15). In such steps the absolute duration of phases of support on one limb increased with the increase in the step cycle duration (*a*: 0.08 to 0.36, *r*: 0.17 to 0.81) (Fig. 5A steps up to 235 ms), while the relative durations were correlated only in some animals (*a*: 0.18 to 0.48 s^{-1}; *r*: 0.31 to 0.58) with the step cycle duration (Fig. 5B). In steps in which the homolateral support was present, (steps between 235 to 430 ms), the duration of the support on one limb began to be shortened by the intervening support on homolateral limbs (Figs 4D and 5). The duration of one-limb support corresponded then to the time interval elapsing between the offset and the onset of the stance phases in the opposite pair of homologous limbs (hind for the

TABLE III

Main characteristics of different support phases occurring in overground locomotion in intact rats

Kind of support phase in the four-limb step cycle	Occurrence (kind of gait)	Characteristic of step	Events determining support duration phase	Support phase duration	Relationships with the step cycle duration	
					Absolute duration	Relative duration
Support on three limbs	only in walk	DF> homologous shift	onset and offset of stances in pair of homologous limbs	DF of the respective limb - homologous shift	correlated	correlated
Support on four limbs	only in walk	DF of hindlimb - diagonal shift> homologous shift of the forelimb	forelimb stance onset and homolateral hindlimb stance offset	DF of hindlimb - diagonal shift - homologous shift of the forelimb	not correlated	not correlated
Support on diagonal limbs	both in run and walk	run steps with no neighbouring supports on 3 limbs	forelimb stance onset and diagonal hindlimb stance offset	DF of hindlimb - diagonal shift	correlated	not or weakly correlated
		walk steps with neighbouring supports on 3 limbs	forelimb stance offset and diagonal hindlimb stance onset	DF of hindlimb - diagonal shift - neighbouring supports on 3 limbs	not or weakly correlated	negatively correlated

TABLE III cd

Support on homolateral limbs	slow run or walk	slow run steps with no neighbouring supports on 3 limbs	hindlimb stance onset and homolateral forelimb stance offset	DF of forelimb - homolateral shift	correlated	correlated
		walk steps with neighbouring supports on 3 limbs	hindlimb stance offset and homolateral forelimb stance onset	DF of forelimb - homolateral shift - neighbouring supports on 3 limbs	not or weakly correlated	negatively correlated
Support on one limb	only in run	steps with no neighbouring support on homolateral limbs	offsets or onsets of stance phases in pair of diagonal limbs	Diagonal shift between the offsets or onsets of stances in pair of diagonal limbs	correlated	not or weakly correlated
		steps with neighbouring support on homolateral limbs	offset and onset of stance phases in the opposite pair of homologous limbs	homologous shift - DF in the opposite pair of homologous limbs	negatively correlated	negatively correlated
Phase of flight	moderate run	lack of support on homolateral limbs	forelimb stance offset and homolateral hindlimb stance onset	homolateral shift - DF of forelimb	negatively correlated	negatively correlated

support on forelimb and *vice versa*), i.e., the difference between the homologous shift and the stance duration (e.g. Fig. 4D and Table II: support on LF: HH1 - DFRH = 0.08; support on LH: HF1 - DFLF = 0.03). In such steps the absolute and relative durations of one-limb supports began to decrease, with *a* and *r* being just of the opposite sign to those for the corresponding supports on three limbs (Fig. 5A and B) (absolute values: *a* -0.47 to -0.07, *r* -0.82 to -0.22; relative values: *a* -1.16 to -0.19 s^{-1}, *r* -0.78 to -0.20). This lasted up to the moment of the apearance of the three-limb support which replaced the one-limb support (steps 320 to 370 ms in Fig. 5).

Phases of flight

Phases of flight, with no support on either limb, were encountered in our animals in short steps (shorter than 235 (No. 2) to 430 ms (No. 4)) characteristic for moderate running, in which the duty factors were shorter than the homologous as well as the homolateral shifts, and hence neither the supports on three limbs nor on homolateral limbs were present. They were observed in 0 (Nos. 3, 6 and 7) to 60% (No. 4) of steps, intervening between phases of support on one limb (Fig. 4E). Their duration was equal to the time interval elapsing between the offset and onset of stance phases in the homolateral pair of limbs, i.e., the homolateral shift minus the stance duration of the respective forelimb (e.g. Fig. 4E and Table II first phase of flight: L1 - DFLF = 0.09). The duration of the phases of flight was correlated with the step cycle duration in an reverse manner as was the duration of the phases of support on homolateral limbs, i.e., decreased with the increase in the step cycle duration (Fig. 5 steps up to 235 ms) (absolute values: *a* -0.51 to -0.29, *r* -0.88 to -0.58; relative values: *a* -1.02 to -0.37, *r* -0.81 to -0.47). In longer steps, the phases of flight were replaced by the supports on homolateral limbs (see above and Fig. 4D).

Table III summarizes the conditions which have to be fulfilled for the appearance of particular support phases and the method of calculating their durations.

DISCUSSION

The analysis of interlimb coordination in overground locomotion in intact rats performed in the present study shows that, within the speed limits used in the present experiments ranging between 10 and 78 cm/s (step cycle duration 685 and 215 ms, respectively), the gait of animals was characterized by a homologous phase shift close to 0.5 and much shorter diagonal than lateral phase shifts. This corresponded to a symmetrical gait with diagonal couplets according to Hildebrandt (1966, 1976). The absolute duration of homologous, diagonal and lateral time shifts was correlated with the step cycle duration in the vast majority of cases, but the respective phase shifts were not correlated. All these phase shifts showed some interlimb and interanimal variability.

The presence of diagonal couplets in rat locomotion has been found by several authors. In the experiments of Hruska et al. (1979), the time lag between the footfalls of diagonal limbs was approximately 10% of the step cycle. Cohen and Gans (1975) reported the lateral shifts ranging between 14 and 50%, which should correspond to diagonal shifts ranging between 36 and 0%. Other authors (Gruner et al. 1980, Molinari and Petrosini 1993) did not assess the diagonal shifts, but from their footfall diagrams values ranging from 0 to approximately 20% could be deduced. These values correspond to those found in the present experiment, in which the mean phase shift in individual animals ranged between 0.0 ± 0.05 and 0.21 ± 0.06. Moreover, our data showing that although the absolute values of the diagonal shifts were correlated, their relative values were not or only weakly correlated with the step cycle duration, stresses the consistency of the fore- hindlimb coordination over a large span of step cycle duration, covering both walking and running (duty factor 0.29 to 0.79). Since during trotting, diagonally opposite legs move more or less in phase, Hildebrandt (1965,1966,1976) called this kind of gait a walking trot (duty factor >0.5) and running trot (duty factor <0.5). Other authors (Howell 1944, Gray 1968) used the term trot for step cycles with a duty factor smaller than 0.5. The latter terminology might be adequate for larger animals, in which the transition from walk to run occurs at a much higher speed than in rats (Heglund et al. 1974). The locomotor speed at which the duty factor reached the 0.5 value, i.e., the transition from walking to running, showed, in our experiments, interlimb and interanimal differences. It ranged mainly between 20 and 40 cm/s, which corresponded to the step cycle duration between 240 and 620 ms (Górska et al. 1998). In the experiments of Hruska et al. (1979), this transition occurred at a speed of 80 cm/s and a step cycle duration of approximately 300 ms (data pooled together from few steps in several animals), while in the experiments of Westerga and Gramsbergen (1990) at a speed of 24 cm/s and a step cycle duration of ca 400 ms. It may be inferred, therefore, that the trot-like synchronization is a dominant feature of rat locomotion because it helps to keep the balance of the body by shortening the support on homolateral limbs, which is unstable especially at slow speed, and increasing the support on diagonal limbs, which is much more stable (Gray 1968). As stressed by Hildebrandt (1966,1976), animals with short legs use this kind of coordination when moving overground at slow rates.

Our results differ from other authors (Cohen and Gans 1975, Hruska et al. 1979, Gruner et al. 1980, Molinari and Petrosini 1993) with respect to the order of footfalls in the step cycles. In the present experiments, the footfall of the hindlimb was followed by a footfall of the diagonal forelimb. This order of footfalls was called by Hildebrandt (1966, 1976) diagonal, in contradistinction to the lateral order of movements in which the footfall of the hindlimb was followed by a footfall of homolateral forelimb, which was described by other authors. The reason for this difference is not known. The lateral order was thought to protect the movements of homolateral limbs from interference. This might be true for locomotion with short homolateral shifts (lateral couplet according to Hildebrandt 1966,1976) in which the footfalls of homolateral limbs are closely spaced in time. However, in the diagonal order and short diagonal shifts (diagonal couplets), the movements of homolateral limbs are separated by relatively long time intervals (lateral shifts) which should also diminish the chances of interference between movements of homolateral limbs. The conditions under which the lateral or diagonal order of limb movement appears deserves further studies.

The analysis of the support patterns in the four-legged step cycles performed in the present study allowed us to investigate how the support patterns and the durations of the support phases changed with the step cycle duration. In walking, the dominant pattern consisted of three-limb support alternating with the support on two limbs, diagonal and homolateral. When the duty factor became equal to or shorter than 0.5, as in running, the pattern of support changed into a support on diagonal limbs alternating with supports on one leg and phases of flight. This change occurred because the support on one limb replaced the support on three limbs, while the phases of flight replaced the support on homolateral limbs. This explains why some phases of support cannot be present simultaneously in the step cycle because they are mutually exclusive.

The analysis of the support patterns has been used as a tool for studying locomotion by several authors (Myubridge 1957, Howell 1944, Gray 1968 and others). The most extensive study was made by Hildebrandt (1976) who differentiated, on theoretical grounds, at least 44 support patterns in symmmetrical gaits. They depended on two variables: the duty factor and the percentage of stride the forelimb was following the hindlimb on the same side. Our data concerning the typical pattern of support in walking and running with short diagonal shifts fit well with his data. However, he paid less attention to changes in the duration of support phases or the way some supports replace others. The presented way of calculating the duration of support phases and their relationships with the step cycle duration seems to fill this gap. They also show the interplay in the duration of various phases of support and can easily explain both the similarities and the differences in support patterns described in the literature. For example, from footfall diagrams shown in the literature on locomotion in rats (Cohen and Gans 1975, Hruska et al. 1979, Gruner et al. 1980, Molinari and Petrosini 1992) one can easily deduce that they concerned walking, because there were no phases of support on one limb nor phases of flight. Moreover, in all these diagrams, the support on both homolateral limbs was much shorter than that on diagonal limbs, due to short diagonal shifts and thus relatively long homolateral shifts. The patterns of support deduced from these footfall diagrams differed, however, from the pattern typical for walking mainly by the presence of four-limb support (Gruner et al. 1980, Hruska et al. 1979), absence of support on two homolateral limbs (Cohen and Gans 1975, Hruska et al. 1979, Gruner et al. 1980) and occasional absence of one three-limb support (Cohen and Gans 1975). All these modifications of the basic pattern of support in walking could be easily explained in view of our data, under the condition that in the definitions of phases of support shown in Table III, the hindlimb would be replaced by the forelimb and *vice versa*. This is due to the lateral order of limb movements described in rat locomotion by other authors, in which if the step cycle is counted from the left forelimb footfall, the diagonal shift (LF-RH) precedes the homolateral shift (RH-RF) and does not follow it as in the diagonal order of limb movement (cf. our Fig.4 with Fig.3 in Hruska et al. 1979). Thus the four-limb support replaced the support on homolateral limbs as in our rats and occurred whenever the forelimb duty factor minus its diagonal shift was greater than the homologous shift between the hindlimbs (Hruska et al. 1979, Gruner et al.1980). The lack of support on homolateral limbs without replacing it by the support on four limbs occurred whenever the hindlimb stance onset was simultaneous with the homolateral forelimb stance offset (Cohen and Gans 1975, Gruner et al. 1980). The lack of one of the three-limb support occurred whenever the stance onset in one limb coincided in time with the stance offset of the other homologous limb, i.e., the duty factor was equal to the homologous phase shift (Cohen and Gans 1975).

This kind of analysis can also be applied to locomotion in other species. For example, it explains the diversity of support patterns observed in walking in intact cats and dogs (for literature see Górska et al. 1993a) as well as the changes in locomotion observed in cats after partial spinal lesions (Górska et al. 1993b). In the latter case, the lesions led to an increase of the diagonal shifts which resulted in an increase of the support on homolateral limbs, partial disappearance of the support on diagonal limbs and appearance of the support on four limbs. Such an analysis seems to reflect changes in locomotion more adequately than the analysis of the shifts only. In the subsequent paper, we shall describe the results of a similar analysis performed in rats with partial spinal lesions.

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