

## Stepping by Chronic Spinal Cats\*

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**Summary.** We investigated the consequences of spinal cord transection in adult cats. All became capable of stepping upon a treadmill. Two of six animals stepped in the first postoperative testing session, 1 week after surgery. It took up to 6 weeks for the others to start stepping.

None of the cats became capable of hindquarter support within 2 months after surgery. There was permanent loss of fore-hindlimb coordination and increased variability in the duration of each step cycle and its components. Coordination between the hindlimbs was also impaired, as signaled by increased variability in the time between foot contacts with the belt. These changes may be due to the loss of timing signals coming down from supraspinal levels to the segmental step generators or – alternatively – to decreased excitability of segmental motoneurons leading to less precise execution of stepping commands from the hypothetical pattern generators.

**Key words:** Spinal cord – Cats – Paraplegia – Stepping – Locomotion

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Complete transection of the spinal cord, under certain experimental conditions, allows the return of rhythmical stepping ability by the hindlimbs. This phenomenon has now been shown in cats, dogs, rabbits, guinea pigs, opossums, ferrets, and rats (Brown 1914; Eidelberg et al. 1977; Forssberg et al. 1974, 1975; Freusberg 1874; Grillner 1973; Grillner and Rossignol 1978; Hart 1971; Hinsey and Cutting 1936; Miller and van der Meché 1976; Ranson and

Hinsey 1930; Shurrager and Dykman 1951). Most of these studies were carried out in chronically spinalized preparations, and the best results were obtained when spinalization was carried out early in life. Also, acutely prepared adult subjects (primarily cats and rabbits) could be made to step rhythmically under the influence of certain drugs, whose mode of action still remains unclear (cf. Grillner and Zangger 1979; Wetzel and Stuart 1976 for reviews of this particular question).

The fundamental importance of these observations is that they provide two of the major arguments for the concept that stepping (and certain other complex rhythmical motor activities, such as scratching) is controlled by segmental “pattern generator circuits”, whose intrinsic organization remains largely unknown. The third argument is that stepping persists or recovers after multiple dorsal rhizotomies, thus excluding the possibility of sequential reflex activation first proposed by Philippon (1905) to account for walking (Grillner and Zangger 1979).

We were interested in exploring whether spinal cord transection in adult cats, chronically prepared, allows the recovery of support and stepping on a treadmill, as it does in kittens, and the properties of such spinal stepping, for later comparison with the consequences of sparing specific sectors of the spinal cord.

### Methods

The subjects of this experiment were six adult mongrel cats. We chose females because it is easier to empty their bladders by expression than it is with males; also, females seem less prone to formation of bladder calculi. Each cat was first adapted to the testing apparatus, which consisted of a rectangular box with one wall made of glass sheet, set over a treadmill belt. The belt moved at 0.22, 0.42 or 0.61 m · s<sup>-1</sup>. The cat's task was to walk upon the belt without gaining or falling behind, for a period of 15 s for a food reward delivered immediately. To increase their motivation

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their entire daily solid food ration was delivered at the end of each training-testing session. Training was carried out until easy, smooth, walking was achieved at all three belt speeds. Filmed records were obtained prior to surgery (see further down).

The animals were spinalized by transecting the cord via a two-segment laminectomy, between T<sub>6-8</sub>. The dura was sectioned together with the cord and a probe was inserted to insure completeness. The gap between the cut surfaces was filled with lightly packed gelatin foam. The wound was closed in anatomical layers. This procedure was carried out under general anesthesia by pentobarbitone (35–40 mg/kg i.v.), with clean technique and under magnification. We treated the animals prophylactically with penicillin (i.m., 100,000 U/day/5 days). They were kept on a soft cage floor; their bladder was expressed twice daily. Passive exercise of each joint was used to keep normal range of motion. The skin was kept clean and coated with lanolin lotion. The animals were first put back on the treadmill 1 week after transection. None became capable of supporting its hindquarters, so that all the postoperative testing was done using a sling with a rope and pulley, which lifted the animals' posterior end approximately to its normal height. Daily retesting at all three belt speeds was continued for a period of 8 weeks, recording the animals' performance on film at least once a week.

At the end of the 8 weeks each cat was anesthetized and the lumbar enlargement (L<sub>3-4</sub>) of the cord was exposed via a laminectomy. Fifty microliters of a horseradish peroxidase solution in buffered saline (15 mg/100 µl, Sigma type VI HRP) were injected into the cord with a micrometer syringe and 30G needle through four tracks, so as to saturate the entire cross section of the cord, at that level, with HRP. Three days later the animals were perfused through the left heart ventricle with heparinized saline and an aldehyde mixture. The CNS was removed, blocked, sectioned, and treated to demonstrate retrograde HRP labeling of neurons on the cephalad side of the transection (Mesulam 1978). The tissue block containing the site of transection was embedded in paraffin and cut sagittally. A modified Bodian stain for cells and axons was used to look for nerve fibers crossing the connective tissue bridge that rejoined the cut ends of the cord.

We filmed the animals' performance with a Super 8 movie camera at 54 fps. and edited out the segments where the film transport system was still accelerating. Footfall diagrams were drawn indicating the position of each paw with respect to the belt surface in each film frame. A continuous line represents "floor" contact (E<sub>2</sub>–E<sub>3</sub>), the blank space to the next dot the *swing* phase (F–E<sub>1</sub>), with elevation of the foot, the dot the onset of floor contact in the next stance phase. A dashed line indicates flexion without foot lift-off. Four consecutive step cycles are shown, aligned by the onset of the right forelimb's stance phase. This method is a slight modification of Hildebrand's (1959). We also measured the duration of step cycles and of their swing (F–E<sub>1</sub>) and stance (E<sub>2</sub>–E<sub>3</sub>) components, and the intervals between the footfalls of different limbs. Such measurements are subject to a possible error close to 20 ms, since film speed was 54 fps.

Joint angle trajectories were determined from the enlarged film projections (Goslow et al. 1973). We also recorded the relative position of pairs of ipsilateral (fore-hind) limbs by reading in a digitizing screen their position in the anteroposterior direction with respect to a fixed body marker (a tattoo mark near the animals' center of gravity). These were plotted by a computer and representative tracings are shown in Figs. 4 and 5. The degree of coupling between the movements of the two limbs was determined by computing the correlation coefficient  $r$  from the same numerical position data. The value of  $r$  could range between 0.00, which indicates no correlation at all, to 1.00 which indicates perfect correlation, whether in or out of phase. Calculation of this coefficient does not require assuming normal distribution of the data (Snedecor and Cochran 1967).

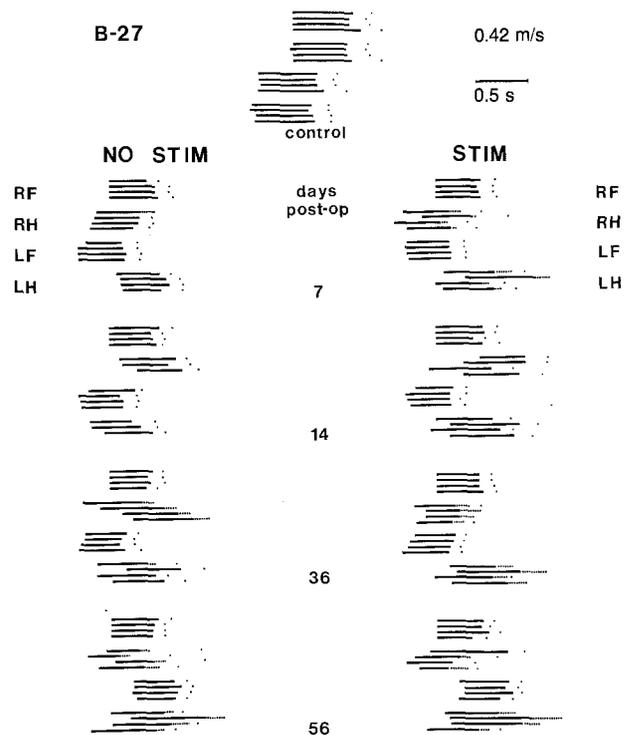


Fig. 1. Footfall diagrams of a cat before and after (7–56 days) midthoracic cord transection. Moderately fast walking speed. "Stim" refers to records taken during skin stimulation (pinching, see Methods). RF: right forelimb, RH: right hindlimb, etc.). Consecutive step cycles aligned by onset of belt contact by right forelimb. Continuous lines: belt contact. Dashed lines: swing phase with belt contact. Space to dot: swing phase and E<sub>1</sub>, foot off belt

## Results

### Anatomical Data

The transections were adjudged to have been complete on the following grounds: By direct observation at the time of surgery, and the use of a curved probe to lift the cord slightly just before cutting it. Second, we found no evidence that axons traversed the scar bridge which rejoined the cut surfaces of the cord, on careful light microscopic review of sagittal silver-stained serial (1/5) sections of the cord (see Methods). Third, we found no HRP-labeled cells in serial (1/20) sections of the cervical cord and brain stem of these cats. We found at least a few labeled neurones in cats where even a minimal number of white matter axons were spared (work in preparation).

### Antigravity Support

None of the cats became capable of "spontaneous" hindquarter support during the period of postopera-

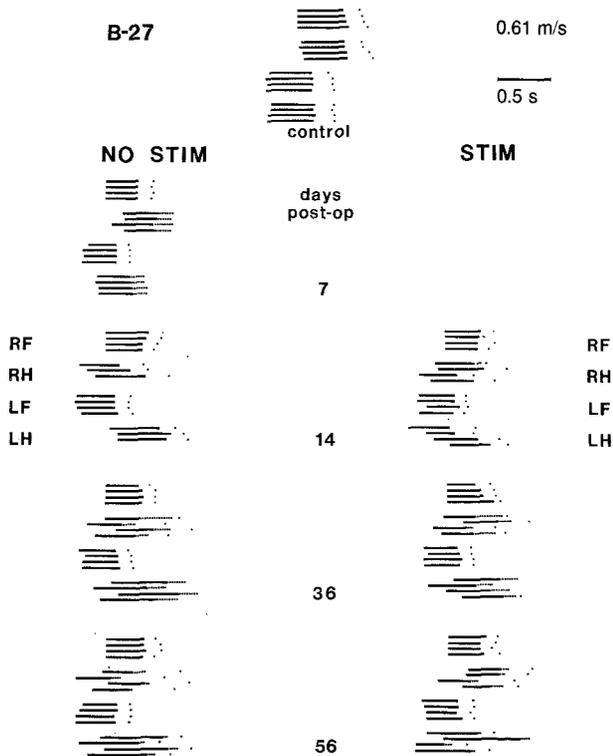


Fig. 2. Same cat and conventions as in Fig. 1, but faster belt speed (brisk walk). Note shortening of step cycles compared to Fig. 1

tive observation. Therefore, all testing on the treadmill was carried out with the sling support, as described in the Methods section. While the animal was on the sling strong stimulation below the level of the lesion, such as pinching the skin at the base of the tail, often resulted in increased extensor tonus in the hindlimbs and in transient support.

#### Forelimb Stepping

The only conspicuous change in the forelimbs was an increase in the rate of stepping for a given belt speed, compared to the preoperative control data. This increase was the result of shortening the stance phase, as was observed by others before with increased belt speed or stronger central stimulation (Arshavsky et al. 1965) (see Figs. 1, 2, 4, and 5).

#### Hindlimb Stepping

The experimental animals were retested daily, after a wait of 1 week after surgery to minimize discomfort. Hindlimb stepping upon the moving belt was observed in the first retest session in two cats. In the other four its onset was delayed, by 2 weeks in one,

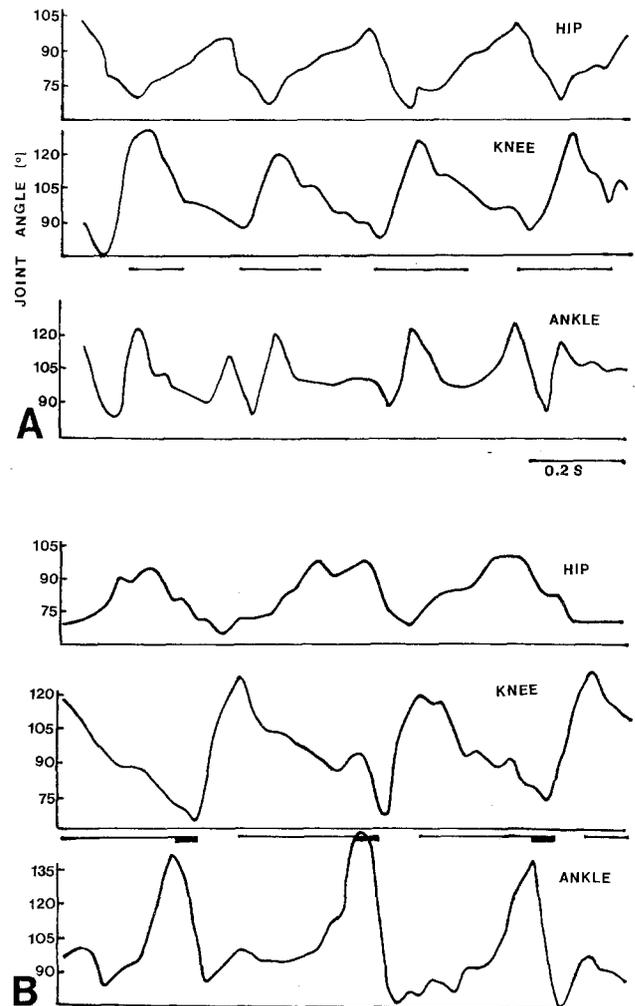


Fig. 3. Joint angle diagrams for the same cat as in Figs. 1 and 2, A = preoperative control, B = 56 days after transection. Lines under knee records: E<sub>2</sub>-E<sub>3</sub> phase. Thick line: belt contact during F-E<sub>1</sub> period

3 weeks in two, and 6 weeks in one. Once it began it appeared in every test session and its characteristics (rate, footfall pattern, joint angles, etc.) improved little from that time on. There was wide variation across subjects in the relative proportion of time in each "trial" when there was stepping. Some did nearly always, some only part of the time. In two of the cats, the same ones which took the longest time to start stepping, the hindlimb movements were rather erratic and disorganized and remained so. Figure 5 contains tracings of the foot trajectories from one of them (B-21) as an example which is clearer than any written description. We did not attempt, in these two cats, to treat the data by analyzing the stride characteristics or the joint angle trajectories as they were clearly unsuitable for such treatment.

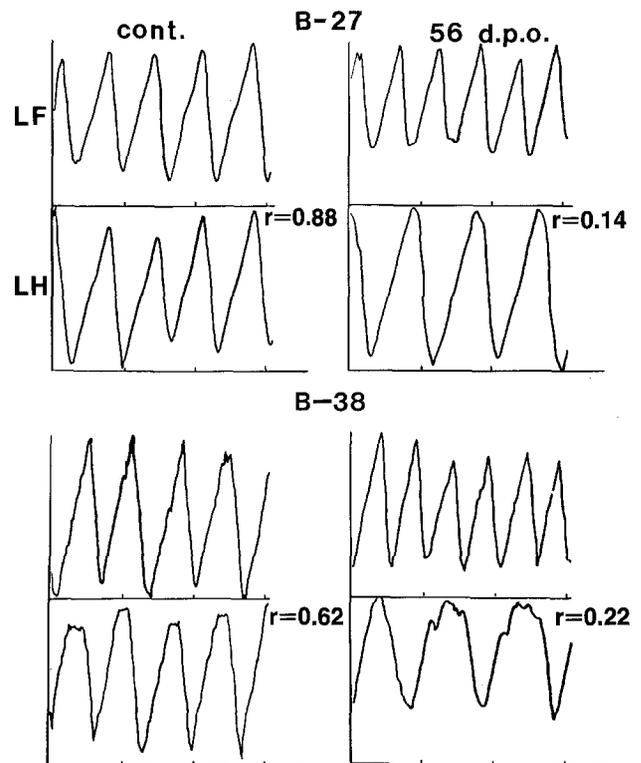
**Table 1.** STEP-CYCLE DATA (Cat B-27) (Time in ms)

	Single Hindlimb (duration)	Belt Speed (m/s)
Control (N = 20 steps)	0.42	0.61
Step cycle	$\bar{X}=730\pm 10$ S.D.	$\bar{X}=603\pm 27$
Swing	$\bar{X}=162\pm 12$	$\bar{X}=187\pm 13$
Stance	$\bar{X}=568\pm 13$	$\bar{X}=416\pm 20$
Post Op (N = 20 steps)		
Step cycle	$\bar{X}=780\pm 134$	$\bar{X}=605\pm 100$
Swing	$\bar{X}=220\pm 112$	$\bar{X}=160\pm 89$
Stance	$\bar{X}=560\pm 100$	$\bar{X}=446\pm 116$
Interval Between Hindlimb Footfalls (ms)		
	Belt Speed (m/s)	
Control (N = 20 steps)	0.42	0.61
Post Op (N = 20 steps)	$\bar{X}=377\pm 22$ S.D.	$\bar{X}=274\pm 27$
	$\bar{X}=223\pm 124$	$\bar{X}=239\pm 118$
Interval Between Fore- and Hindlimb Footfalls (same side)		
	Belt Speed (m/s)	
Control (N = 20 steps)	0.42	0.61
Post Op (N = 20 steps)	$\bar{X}=40\pm 26$ S.D.	$\bar{X}=30\pm 30$
	$\bar{X}=260\pm 123$	$\bar{X}=150\pm 110$

Data on the timing step cycles, taken from films of the same cat as Figs. 1–3; no skin stimulation. Note the marked post-transection increases in standard deviation of the means

The stepping movements in each hindlimb generally resembled those observed preoperatively, as illustrated in Figs. 1–3. There were some consistent differences, however, which involved the duration of the overall step cycle and its swing and stance components. The mean value of these measures was not significantly altered but their variability – expressed as the standard deviation of those means – increased several fold, as shown in Table 1. Our testing situation tended to reduce this variability compared to overground walking (Wetzel et al. 1975; Goslow et al. 1973; English 1979). Another difference was that in the intact cat the swing phase is carried through to the beginning of extension with clear liftoff of the paw from the belt. Postoperatively the paw was dragged forward over the belt and it was not lifted at all or only just before the  $E_1$  phase began (Philipsson 1905). This is shown in the footfall diagrams of Figs. 1 and 2 as dotted line and in Fig. 3 as a thickened line at the end of the stance phase markers.

Finally, one of the more consistent features of “normal” walking in the cat is the close in-phase correlation between the movements of the knee and ankle joints (Goslow et al. 1973). This correlation was disturbed by the transection, as can be seen in the illustrative example in Fig. 3.



**Fig. 4.** Computer-generated tracings of left fore- and hindlimb trajectories (foot targets) in the anteroposterior axis only. Two spinal cats, before and after transection. *r*: correlation coefficient for each fore-hindlimb pair (see Methods, last paragraph). *Up* = forwards movement with respect to mid-body marker, *down* = backwards movement. Roughly equivalent to swing and stance phases. Time marks: 1 s

#### Hindlimb-hindlimb Coordination

This is described in Table 1 as the differences in time between the onset of belt contacts ( $E_1-E_2$ ) by each hindlimb. As with the individual step cycle timing data, the mean values for this interval were not greatly altered by spinalization but their standard deviations increased several fold.

#### Fore-hindlimb Coordination

We analyzed it in two ways: One by measuring the mean time difference between the onset of floor contact by the forepaw and hindpaw on the same side, and its variability. These measurements are listed in Table 1 and show that both the mean intervals and their standard deviations were altered by cord transection, the latter increasing several fold after it. Figures 4 and 5 contain representative samples of computer plots of the trajectories (in the anteroposterior direction only) of a fore- and a

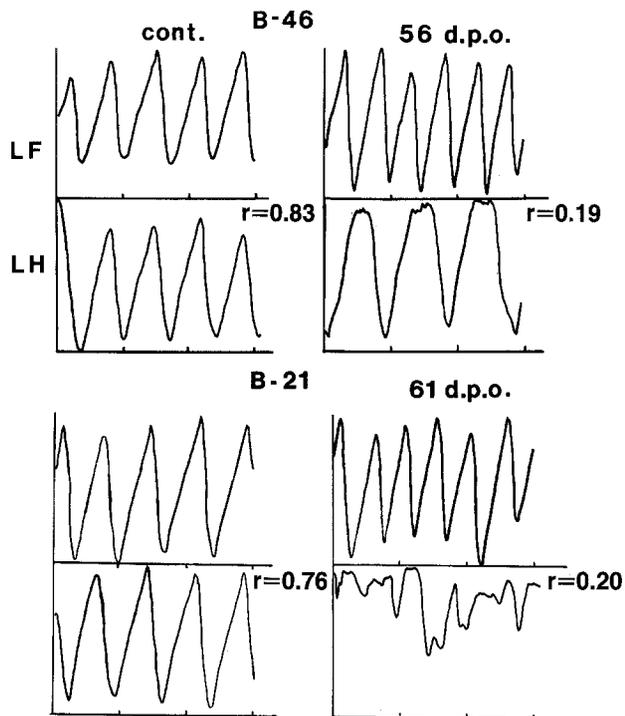


Fig. 5. Same as Fig. 4. Note the rather irregular movements of the hindlimb in cat B-21, compared to the other cats'

hindlimb in the same side, in four of the cats. It is obvious from simple inspection that there was no phase coupling after surgery, and this was borne out by the correlation coefficient values noted in each graph (see Methods).

## Discussion

We will discuss some of the evidence concerning locomotion in spinal cats under three arbitrarily separate categories: support, stepping, and interlimb coordination.

With regard to *support* for the hindquarters our observations differ from those by Shurrager and Dykman (1951) and from those made in Grillner's laboratory (Forssberg et al. 1974, 1975; Grillner 1973; Grillner and Rossignol 1978) in that most of their animals were capable of such support and only required lateral stabilization. Their cats had been transected soon (1–4 weeks) after birth. Ours, transected in adulthood, could support their hindquarters while strongly stimulated by the treadmill motion and skin pinching. This suggests that our cats had a potential capacity for support which was not expressed as well as in kittens. We transected our cats at a higher spinal level than they did (mid- vs. low-thoracic), but this is not sufficient to account for the

differences since the critical muscles for support are innervated from below L<sub>1</sub>. The usual explanation for differences between early and later lesions is that the younger CNS has greater "plasticity". Lacking a reasonably agreed upon meaning for such a term, or an anatomical substrate for it, it is not possible to accept or exclude it as an explanation (Discussion in Eidelberg and Stein 1974). Controlled experiments are needed to test the possibility that training may influence standing and stepping differently in young and adult animals.

The return of rhythmical *stepping* after transection in cats and dogs is very well documented in the literature since Freusberg's report of 105 years ago (1874). It was the basic observation for the now currently accepted view that spinal "step generator circuits" govern the basic sequence of limb movements during locomotion, under supraspinal gating and modulatory control (Brown 1914; Grillner 1975; Grillner and Zangger 1979; Miller and van der Meché 1976; Shik and Orlovsky 1976; Wetzel and Stuart 1976). As Forssberg et al. (1974 and 1975), Grillner (1973) and Grillner and Rossignol (1978) have noted, the main components of the step cycle are found in the spinal cat placed on a treadmill. There was even gait conversion (walk→trot→gallop) when the belt speed was changed. Our observations agree generally with theirs in that four of six of our cats did exhibit a quasi-normal sequence of joint angle trajectories during each step cycle. The differences from normality in these animals appeared as substantial increase in the variability in the duration of hindlimb step cycles and of their swing and stance components, and also as a change in the in-phase coupling between knee and ankle joint motions. These differences lead us to conclude that the timing of the sequences of muscle contractions in each step may be basically determined by the segmental "step generators", but that it is importantly modulated or "tightened" by suprasedgmental influences in addition to segmental reflex inputs (Grillner and Rossignol 1978; Grillner and Zangger 1979; Miller et al. 1975).

*Interlimb coordination* refers to the relative timing of the step cycles by both hindlimbs and by the fore and hindlimbs. The first is presumably mediated by segmental crossed propriospinal connections (Brown 1914; English 1979; Grillner 1975; Kulagin and Shik 1970; Miller and van der Meché 1976; Miller et al. 1975). It should, therefore, not be greatly disturbed in the hindlimbs by a midthoracic transection. However, a substantial increase in variability in footfall intervals was found by us, as described in Results, even though the basic alternating pattern was preserved. The most parsimonious interpretation is that this reflects the increased varia-

bility in stepping by each hindlimb. Fore-hindlimb coordination was lost altogether, as might be expected from the severance of all linkages between the cervical and lumbar enlargements of the cord (Coss et al. 1978; English 1979; Miller and van der Meché 1976). What is interesting, and not readily explained, are the changes in the stepping rate by the forelimbs after transection: In effect the forelimbs acted as if the belt speed was higher, so that the stance phase was shortened relative to its preoperative duration for the same belt speeds (Arshavsky et al. 1965) (Figs. 1, 2, 4, and 5). Since the hindquarters were lifted above "ground" this effect could not be a response to increased load upon the forelimbs. Shik and Orlovsky (1965) studied the consequences of placing a dog upon a treadmill so that only the forelimbs contacted the belt. Contrary to our spinal cats the stepping rate was unchanged. This suggests that the increases we saw are a consequence of spinalization, but whether or not this is related to the Schiff-Sherrington phenomenon remains to be investigated.

We conclude that spinal cord transection in the adult cat leads to relatively more severe functional losses than the same procedure does in kittens operated before the time they are capable of spontaneous locomotion. Why this should be so cannot be adequately explained by differences in "plasticity", at least until that term acquires some specific meaning. While most of our cats exhibited rhythmical hindlimb stepping most of the time, in two of them the rhythmicity was less obvious. Even in the "better" animals, the precise timing of step cycles seen in treadmill locomotion was disrupted. This could reflect the removal of suprasedgmental timing influences acting as "clocks", or a reduction in segmental neuronal excitability leading to "jitter" in the commands from the central pattern generators.

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