

Modulation of Ipsi- and Contralateral Reflex Responses in Unrestrained Walking Cats

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SUMMARY AND CONCLUSIONS

1. The modulation of reflex responses in up to 10 simultaneously recorded hindlimb muscles was studied in unrestrained cats walking on a treadmill. Single electrical shocks of various strengths were applied to different skin areas of the hindlimb at different times of the step cycle while the resulting EMG responses were sampled and analyzed.

2. Two excitatory response peaks (P_1 and P_2) at a latency of about 10 and 25 ms, respectively, were seen in all flexors examined (sartorius, semitendinosus, tibialis anterior, extensor digitorum longus). Stimulation of most skin areas was effective but responses were most easily obtained from stimuli applied to the foot or ankle. During the step cycle there was a marked modulation of the amplitudes of the responses, especially the P_2 responses, which grew larger toward the end of stance when a maximum was reached, followed by a steady decline throughout swing. This pattern was very similar for various flexors, although these muscles differed considerably in their normal EMG activity pattern during walking.

3. Flexor responses were absent when the same stimuli were applied during the early stance phase. Instead, inhibition of the ongoing EMG activity was seen at a latency of 10 ms or less in all extensors examined (semimembranosus, quadriceps, soleus, gastrocnemius medialis, flexor digitorum longus). The inhibition was followed by a late excitatory peak (P_3) at about 35-ms latency in all extensors except soleus.

4. Certain stimulation sites yielded exceptions to the above patterns. Stimulation of the skin area innervated by the sural

nerve yielded larger and earlier MG excitatory responses as compared to stimulation of other skin areas. Activation of the plantar surface of the foot often failed to elicit P_2 responses in the hip flexor sartorius, which showed inhibition instead.

5. In the hindlimb contralateral to the stimulus, excitatory responses occurred both in flexors and extensors at a latency of 20–25 ms. The pattern of modulation of these responses was similar to the ipsilateral modulation of P_2 flexor and P_3 extensor responses. Soleus failed to show a crossed response.

6. The data indicate that flexor and extensor responses differ both with respect to their latency and to their correlation with the ongoing EMG activity. It is concluded that these stimuli do not demonstrate reflex reversal in the strict sense in the normal walking cat but that there is modulation of transmission in a flexor excitatory and extensor inhibitory pathway, possibly by the flexor part of the spinal locomotor oscillator. In addition, there are some specialized flexor inhibitory and extensor excitatory pathways. The slow soleus muscle does not seem to be excited through these pathways.

INTRODUCTION

For a reflex response elicited in a moving limb to be appropriate, its amplitude has to be determined not only by the locus and the intensity of the stimulus but also by the phase of the ongoing movement. In the case of mammalian walking movements, for example, strong activation of flexor muscles in the withdrawal reflex would be inappropriate if it occurred during the period of the step cycle during which the stimulated hind-

limb is supporting the weight of the animal. The modulation of reflex amplitudes in the course of a movement may be due to several mechanisms. The net membrane potential of motoneurons will determine the degree of recruitment of a muscle pool by a given afferent input. In principle, this mechanism alone might explain "phase dependent reflex reversals" (14) in which a given skin stimulus elicits excitatory responses in flexors during swing and in extensors during stance. However, the pattern of reflex modulation during the step cycle is often poorly correlated with the ongoing rhythmic EMG activity, suggesting an additional premotoneuronal gain control of reflex pathways (14). Direct evidence for such reflex gating was obtained by Andersson et al. (1) who showed that the modulation of exteroceptively induced postsynaptic potentials (PSPs) was largely independent of the temporal variations of the membrane potential during "fictive" step cycles.

Recently, the study of phasic reflex modulation has been extended to walking cats with intact CNS (12, 13, 20, 28). It was found that nonnoxious cutaneous stimuli elicited large flexor responses when given at end stance and during swing, while extensor inhibition prevailed for stimuli given during the early-stance phase. Although the behavioral correlates of various responses were described in considerable detail (13, 28), little is known about the participation of individual muscles and the timing of these reflexes and about possible differences among skin areas. From experiments on immobile decerebrate cats, it is known that cutaneous reflexes have "local sign" (21) and that the response to the same stimulus may differ from one muscle to another, even within a pair of synergist muscles (17). Investigation of cutaneous reflexes is further complicated by the characteristic variability of these reflexes due to their polysynaptic pathways. Therefore, a large body of data is needed on many muscles using different kinds of stimuli.

In the present study, the reflex EMG responses to single shocks, applied to one of several skin locations, were compared in up to 10 simultaneously recorded hindlimb muscles of otherwise intact cats walking on a treadmill. An automated analysis allowed

for the quantitative measurement of both excitation and inhibition at various intervals following a large number of stimulus trials.

The data indicate that the modulation of the reflex responses in different flexors is very similar, showing a peak amplitude at end stance. Hindlimb extensors, however, show some marked differences in their reflex responses, both to ipsilateral and contralateral stimulation.

METHODS

Length gauges and bipolar EMG electrodes were implanted in several muscle and skin sites in one or both hindlimbs of six cats. Three of these cats were used for another study as well (18), and a detailed description of the implantation technique can be found there. The electrode pairs had exposed tips of 1 mm, spaced 3 mm apart. A short distance between the tips was chosen to obtain selective recording and stimulation of the muscle studied. The stimuli presumably activated both local receptors and nearby afferent as well as efferent nerve fibers. The electrodes were buried as deep as possible in the muscle or skin and kept in place with sutures.

Most of the electrodes were implanted in the left hindlimb (hereinafter referred to as ipsilateral) in the following locations: medial gastrocnemius (MG, $n = 6$); semitendinosus (ST, $n = 5$); extensor digitorum longus (EDL, $n = 3$); semimembranosus, pars posterior (SM, $n = 2$); sartorius, pars lateralis (SA, $n = 2$); flexor digitorum longus (FDL, $n = 2$); tibialis anterior (TA, $n = 2$); soleus (SOL, $n = 2$); vastus lateralis (QA, $n = 1$). In the right (contralateral) limb, the electrodes were implanted in co-MG ($n = 2$) and co-TA ($n = 2$).

Some of the presently investigated muscles are bifunctional: i.e., SA (hip flexor-knee extensor), ST (hip extensor-knee flexor), SM (hip extensor-knee flexor), MG (knee flexor-ankle extensor). To simplify the description, however, these muscles will be classified according to their participation in the flexor reflex (23). Hence, SA and ST are considered flexors and SM and MG extensors. Figure 1 shows a typical step cycle and the EMG patterns recorded in these muscles by electrodes of this design. At these slow gaits, the typical "double burst" pattern of the bifunctional thigh muscles is not consistently present in each step cycle but contributes to the averages of many step cycles, as described below.

Skin electrodes were implanted in the ipsilateral hindlimb on the lateral surface of the ankle ($n = 5$), dorsum of the foot ($n = 2$), plantar

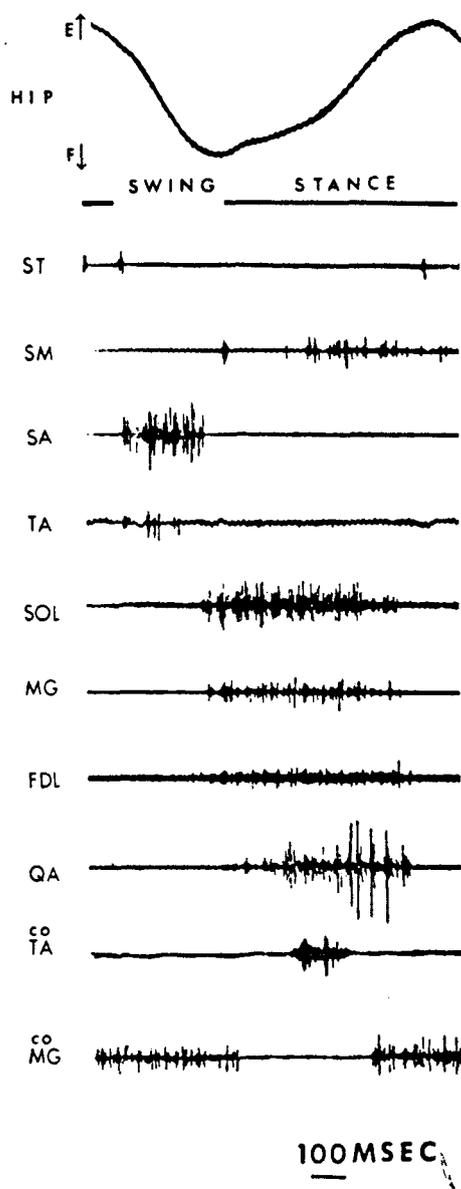


FIG. 1. EMG activity in 10 simultaneously recorded hindlimb muscles during a step cycle. Note the absence of cross talk between flexors and extensors. Hip excursion, recorded with an implanted length transducer, and periods of foot contact, analyzed from single-field video records, are given at the top of the figure for the left hindlimb. Peak hip flexion and extension correspond to hip angles of 70 and 110°, respectively (as measured from video). For abbreviations, see METHODS.

surface of the foot ($n = 1$), skin overlying the patella ($n = 1$), the shin ($n = 1$), and the popliteal fossa ($n = 1$). The paired wires were drawn into the subcutaneous tissue and sutured in place.

The actual electrode placements in skin and muscle were confirmed by postmortem dissection. Electrode impedances were checked daily and were found to be stable after the first postoperative days.

Length/joint angle gauges implanted in the ipsilateral limb included a hip gauge (anterior iliac crest to distal femur), a knee gauge (mid-femur to midtibia), and an ankle gauge (midtibia to calcaneus). The leads from electrodes and gauges were passed subcutaneously to a saddle connector. EMG signals were processed using Bak Electronics MDA-1 differential amplifiers and length-gauge signals using ABI-1 AC bridge amplifiers.

The cats were trained to walk on a treadmill at a uniform speed of 1–1.5 steps/s (0.4–0.7 m/s) for a food reward while single stimuli of 1 ms duration were applied once every 4 s to one of the pairs of skin or muscle electrodes. The movements of the cats as well as monitor lights that flashed when stimuli were being delivered were recorded on videotape by two cameras, one on each side of the treadmill. EMG, joint angles, stimulus intensity, and timing information were recorded on 14-track FM tape (DC–2.5 kHz).

Two of the cats were anesthetized after 2 wk of stimulation experiments and the superficial peroneal and sural nerves were exposed. Evoked potentials were recorded from these nerves and signal averaged while stimulation was applied through the implanted electrodes to the skin sites supplied by these nerves. After determination of the conduction velocity of the afferent fibers stimulated with standard pulse parameters, the two nerves were cut. Postoperatively, the stimulation experiments during walking were repeated to evaluate the effect of the skin denervation. After these control experiments, the cats were anesthetized for the final acute experiment in which all the previously used stimuli were tested by signal averaging evoked potentials in ventral and dorsal roots to determine the conduction velocities of the stimulated fibers.

Runs lasting 8–10 min yielded samples of at least 108 stimulus trials applied at different random times within the step cycle. The reflex responses in a given muscle for these 108 trials were analyzed using a PDP-12 computer, which was programmed to sample the integrated EMG signal (see below) over periods lasting 8 ms, one just before (control) and three at intervals just after the stimulus (responses). The use of the EMG value just preceding the stimulus as the control level against which each reflex response was compared facilitated reflex quantification in muscles with irregular activation patterns. Phase errors were minimal since the time between the beginning of the control period and end of the

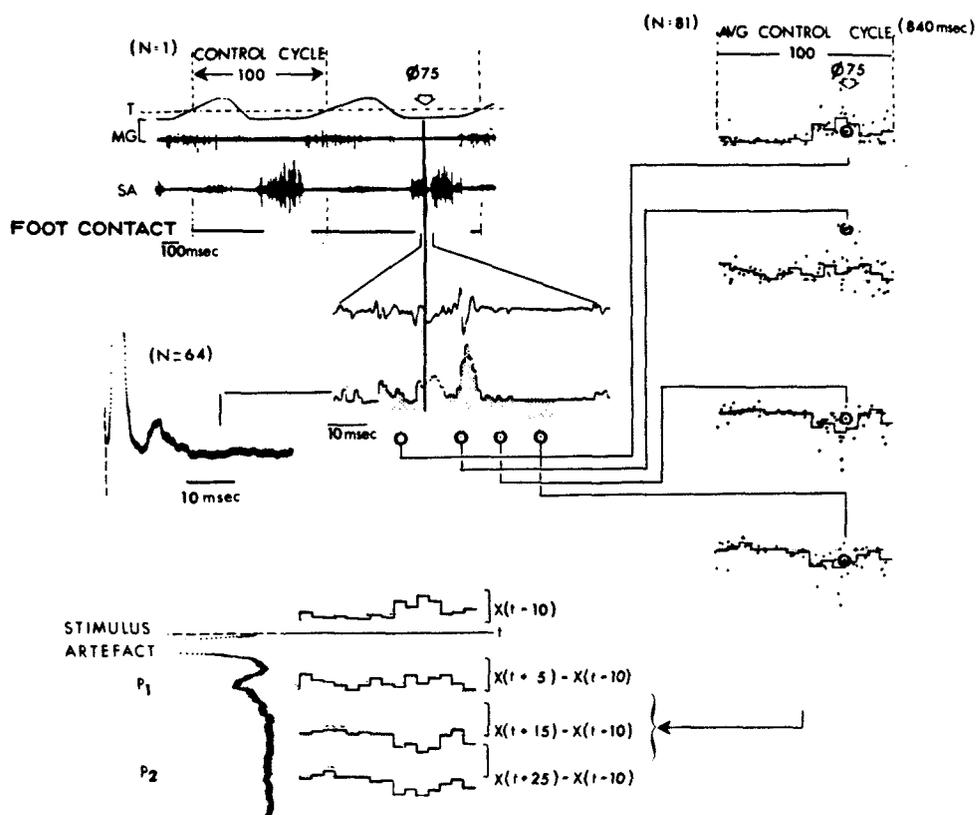


FIG. 2. EMG reflex responses in the lateral sartorius following single 1-ms shocks of 20 mA applied to the plantar surface of the foot during slow walk. Single stimulus trial is given at the left top; methods of analysis for 81 trials during an 8- to 10-min run are given at the right of the figure and summarized at the bottom. $\phi 75$ indicates that the stimulus occurred at a phase of 75% of the step-cycle duration.

response measurement was always under 50 ms, less than 5% of step cycle.

The different stages of the analysis are summarized in Fig. 2. The EMG signal was full-wave rectified and a specially designed pulsed integrator (Bak Electronics PSI-1) computed the area under the EMG curve in 1-ms bins, which were independent of preceding bins (2). The computer sampled this EMG signal (four stippled areas in Fig. 2) after it passed an analog delay line (Bak Electronics AD-6) set at 12 ms (3). The prestimulus sample $X(t-10)$ was then subtracted from the poststimulus samples $X(t+5)$, $X(t+15)$, $X(t+25)$ and, for some, $X(t+35)$ to obtain the net reflex component of the EMG.

The computer also rearranged the randomly delivered stimuli according to their position with respect to the phase of the step cycle, taking the preceding control cycle as a reference (see Fig. 2). The timing of the step cycles was determined using a muscle with a regular EMG pattern (SOL or MG). This EMG was rectified and integrated with a 0.5-s time constant to

give a very smoothly fluctuating signal, which was processed through a variable-threshold Schmitt trigger. The level of EMG activity reached around the moment of ipsilateral footfall was set to generate a step-cycle onset signal read by the computer, which continually monitored each step-cycle duration. When a stimulus pulse occurred, the computer determined its phase in the step cycle by comparing its time of onset to the duration of the previous step cycle.

Finally, the resulting values for the four samples were displayed in four plots (circled dots on the right of Fig. 2) in which the abscissa represents the phase of each stimulus application and the ordinate gives the magnitude of the control and net reflex responses displayed using the same scale. For the example in Fig. 2, the first sample following stimulation was larger than the control EMG sample, resulting in a positive y value on the second plot (circled data points). On the other hand, the amplitudes of samples $X(t+15)$ and $X(t+25)$ were smaller

TABLE 1. *Effect of stimulus intensity on latencies of P₁, P₂, and P₃ responses*

Stimulus Site	Stimulus Intensity, mA	P ₁ (ST), ms	P ₂ (ST), ms	P ₃ (QA), ms
Dorsum	5	11	28	31.5
	10	10.5	24	35
	20	8	23.5	35
Ankle	2.5	9.5	28	29.5
	5	9.5	26	31.5
	20	7	24.5	37.5

P₁, P₂, and P₃ responses are averages. $n = 64$.

than the control, thus yielding negative values in the corresponding plots. The positive and negative values are a measure of the net excitation or inhibition induced by the stimulus since the control plots use the same vertical scale as the net reflex plots.

Similar analysis was done on each of the trials sampled, but an editing routine rejected all trials in which the preceding "control" step cycle had a duration outside the range of $\pm 15\%$ of the mean step duration, eliminating irregular step sequences. In Fig. 2, 81 of the 108 trials passed the 15% criterion. The 81 points in each graph were collected in 15 bins; by averaging the values falling in each bin, a bar diagram was constructed, as seen in Fig. 2 (right side and bottom). The profiles of these bar diagrams indicate that there was a net excitatory reflex effect at a time ($t + 5$ ms) and a net inhibitory reaction at a time ($t + 15$ ms) and ($t + 25$ ms) for stimuli applied in the last 40% of the step cycle, during which the examined muscle (SA) was normally active.

In addition, each experimental run was analyzed for the average reflex response elicited by a given stimulus regardless of step-cycle phase. For this purpose, the first 64 of the 108 rectified EMG responses were summed using a Nicolet 1070 signal averager (see left side of Fig. 2). The resulting average response was used to measure response latencies (Table 1). When placed vertically alongside the bar diagram, the averaged response corresponds to the time of the respective poststimulus sampling periods. The stippled bands connect each bar diagram with the corresponding segment of the average response. The scale for the four bar diagrams in each set was always identical, but the overall scale for each set was individually adjusted to avoid overlap of the different traces. This caused the control bar diagrams to be rather small when large reflex responses occurred.

In total, 466 individual muscle "runs" were recorded (response of a single muscle to at least

108 presentations of a given stimulus); 116 were computer analyzed.

Statistical significance

To test whether the reflex excitations and inhibitions were significant, it was not sufficient to compare individual bins of the bar diagrams since the number of data points within each bin was small and variable (between two and six). Therefore, groups of four bins were lumped together. For example, by applying the Student t test on the 18 data points situated nearest the point of foot lift in Fig. 2 (four bins), it was found that both the excitation (second line) and the inhibition (third and fourth line) were significantly different from control values at the 0.05 level. By repeating this procedure for several four-bin groups, it was found more generally that changes over a period of four bins were significant ($P < 0.05$) whenever their average values differed from the control levels by 50% or more.

RESULTS

Stimulation of distal skin sites of the hind-limb such as the plantar or dorsal surface of the foot or the lateral surface of the ankle were much more effective in eliciting reflex responses than proximal skin sites such as the ventral or dorsal surfaces of the knee. Despite such regional differences in threshold, the responses were very similar qualitatively except for some special reflex effects in certain muscles following stimulation of the plantar surface of the foot or the lateral surface of the ankle.

We first consider the reflex events as lumped together, without considering the gating imposed by the step cycle. In the ipsilateral flexors (SA, ST, TA, and EDL) the responses consisted of two peaks, a first peak (P₁) at a latency of 10 ms and a second peak (P₂) at a latency of 25 ms (Fig. 3, Table 1). The responses in the ipsilateral extensors were characterized by an early inhibition (from 10 to 25 ms) followed by a late excitatory peak (P₃) at a more variable latency of about 35 ms. Responses with P₃ latency were most consistently found in QA but could occur in other extensors as well. Following stimulation of the dorsum with 5 mA, for example, responses with minimal latencies of 34, 31.5, and 31.5 ms were found in SM, QA, and MG, respectively. Contralaterally, the responses in flexors and extensors consisted of a single

excitatory peak at the same or slightly shorter latency than the ipsilateral P_2 (Fig. 3).

Ipsilateral responses

The modulation of reflex responses throughout the step cycle is shown in Fig. 4 for six simultaneously recorded muscles from the hindlimb in which single shocks of 2.5 mA were applied to the skin of the lateral surface of the ankle. The normal EMG activity of these muscles during walking (top trace of each set of bar graphs) is derived from the control EMG levels preceding each stimulus presentation. A single, unstimulated control cycle for these muscles is given in Fig. 1. The hip flexor SA and the ankle flexor TA start to be active at the end of stance and their activity persists throughout swing. SM, which is mainly a hip extensor, is active during most of stance, as are the knee extensor QA and the ankle extensors SOL and MG. The averaged responses of these muscles are given at the

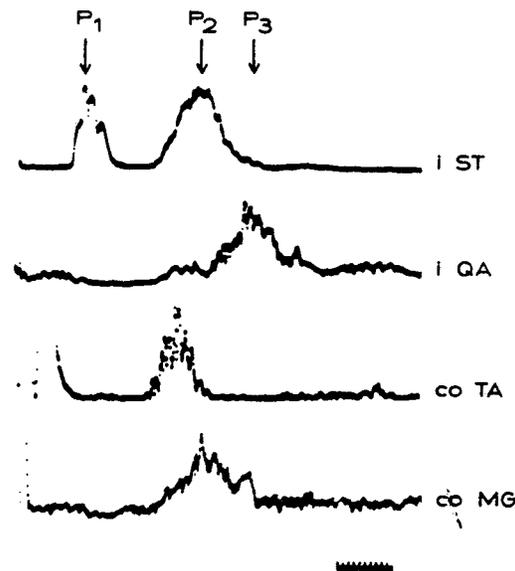


FIG. 3. P_1 , P_2 , and P_3 reflex responses following 10 mA-stimuli applied to the skin of the dorsum of the foot during walking. The P_1 and P_2 peaks are seen in the ipsilateral semitendinosus (i ST), while a single late peak (P_3) appears in the ipsilateral quadriceps (i QA). Contralaterally, a single response at P_2 latency is evident in the tibialis anterior (co TA) and the gastrocnemius medialis (co MG), both of which show some stimulus artifact as well as at the beginning of the trace. The EMG signals were rectified and averaged for 64 presentations at random points in the step cycle. Time calibration: 10 ms with 1-ms divisions.

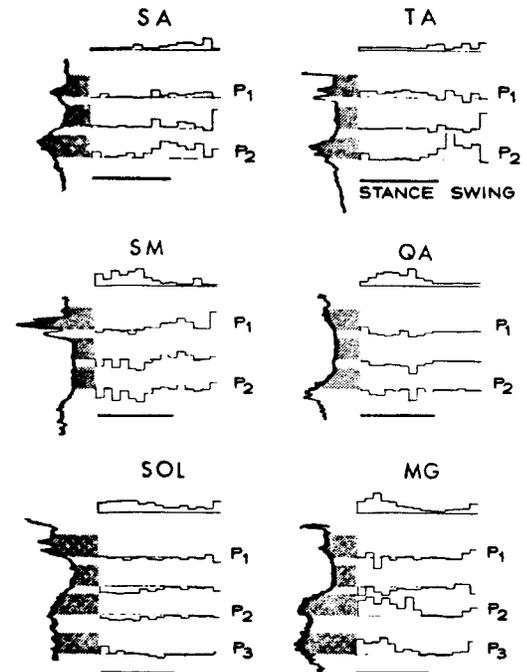


FIG. 4. Modulation of reflex responses following 2.5-mA shocks applied to the skin innervated by the sural nerve. Note the P_1 response in SM and the P_2 responses in SA and TA during late stance and early swing. During yield, an inhibition is seen in all extensors (SM, QA, SOL, and MG), while MG shows an additional large, long-lasting excitatory response both in the 25- and in the 40-ms bar graphs (P_2 and P_3). The modulation of the P_1 responses in TA is partly obscured by the inclusion of the tail of the stimulus artifact in the sample. The duration of stance and swing was determined by measuring the times of foot placing and lifting on video records for 10 unperturbed step cycles.

left of each bar diagram, and one recognizes the usual P_1 and P_2 responses in the flexors as well as the inhibition and late recovery in extensors.

The distribution of the responses during the step cycle is shown below the top control trace in three or four lines, representing the 8-ms EMG integrals taken at 5-, 15-, 25-, and (SOL and MG only) 35-ms periods following stimulation (see METHODS). The P_1 and P_2 responses of SA and TA, as well as the P_1 response of SM, appeared mostly at end stance and throughout swing. During stance, all extensors (SM, QA, SOL, MG) showed a period of inhibition that was maximal at a latency of 15 ms (third trace). In MG, the inhibition was followed

TABLE 2. P_2 responses during walking

Stimulus Site		Stimulus Intensity, mA	SA	TA	ST	SM	QA	MG	SOL	
Early swing	Dorsum	5	+	+	+	(+)				
		10	+	+	+	(+)			(+)	
		20	+	+	+	(+)				
	Postdenervation	20								
		Ankle	2.5	+	+	+				
			5	+	+	+				
			8.5	+	+	+	(+)			
	20		+	+	+	(+)		+		
	Early stance	Dorsum	5	NT	NT					NT
10			+	+	+	+			-	
20			+	+	+	+			-	
Postdenervation		20				-		-	-	
		Ankle	2.5	+					+	
			5	+					+	
8.5								+		
20			+					+	-	

Responses with P_2 latency in seven simultaneously recorded muscles during early swing and early stance. The first four bins of swing and stance for each run (108 stimulus trials) are summed and compared to the control bins. Increase or decrease over 50% (see METHODS) are indicated as + and -, while smaller changes are given as blank spaces. NT, not tested. P_2 responses that are presumed to be due to EMG cross talk are shown in parentheses.

by an excitatory response at P_2 and P_3 latency. A late excitatory response at P_3 latency could appear in other extensors such as SM and QA as well, but never in SOL (Fig. 4).

Pure flexors and extensors were clearly divided on the basis of their different reflex responses. Bifunctional muscles such as SM, however, exhibited both inhibition during stance and P_1 excitation during swing. The P_1 was not due to pickup from the neighboring ST since the P_1 of ST was small and differently modulated. Moreover, the control trace of SM shows no activity at end stance when the simultaneously recorded ST was maximally active. On many occasions SM was found to exhibit P_2 responses at end stance, but cross talk with ST could not be excluded in these instances.

To judge the amplitudes of the various excitatory and inhibitory responses, one has to compare the height of the various reflex bars in Fig. 4 with the corresponding control EMG bars on the top trace. A change of 50% or more for the total of four consecutive bins was found to be statistically significant (see METHODS and Table 2).

Behaviorally, the P_1 , P_2 responses in the flexors were correlated with a brisk flexion movement, recently described in detail by Forssberg (13). During early stance, the extensor inhibition resulted in a visibly increased yield of the stimulated hindlimb (12, 13).

Stimulus intensity. The reflex responses described in Fig. 4 were mostly due to activation of cutaneous afferents since denervation of the skin area involved (by cutting the sural nerve) abolished the responses following application of the same 2.5-mA stimuli. Measurement of the evoked potentials in the sural nerve before cutting revealed that 4.8 mA was the threshold for the activation of A- δ -fibers (conduction velocity of 10 m/s). Hence, it appears that the presently described reflex responses require stimulation of only the larger, myelinated mechanoreceptive afferents. An increase in the stimulus strength did not alter the results fundamentally (see Table 2). As seen in Fig. 5, the step-cycle distribution of responses at P_2 latency in either SA or MG did not change when stronger stimulus cur-

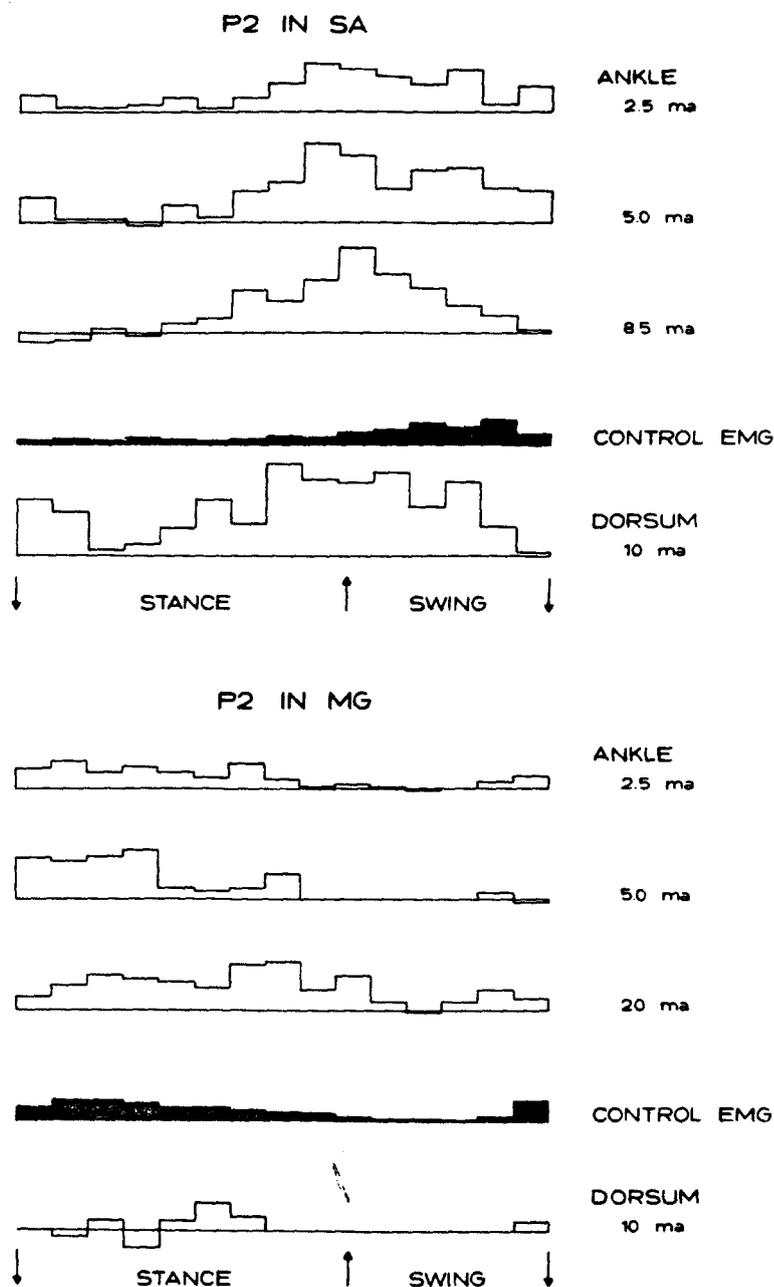


FIG. 5. Comparison of P_2 responses in SA and MG for different intensities of stimuli applied to the skin on the lateral surface of the ankle (ankle) or on the dorsum of the foot (dorsum). The control EMG is the mean of the four control EMGs for the four conditions after appropriate scaling. Downward and upward arrows: foot placing and lifting.

rents were used, although there was a tendency for the responses to grow larger and to be spread over a larger section of the step cycle. The same was true for the P_1 re-

sponses and, in addition, it was found that P_1 responses kept on increasing in amplitude with increases in stimulus intensity long after P_2 responses stopped growing. Note

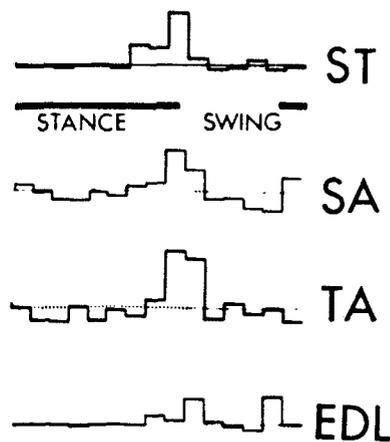


FIG. 6. Step-cycle distribution of P_2 EMG responses of ST, SA, TA, and EDL following 2.5-mA stimuli applied through the electrodes implanted in FDL. Stimulation of this muscle yielded results identical to results obtained with skin stimulation.

that in SA, the P_2 responses were distributed in a pyramidlike fashion with a peak around foot lift. The shape of this pyramid differed completely from the control SA EMG activity, which exhibited a smooth increase toward the end of swing. Individual presentations sometimes failed to show clearly the P_2 response maximum at end stance (Fig. 5), thereby emphasizing the need to analyze a sufficiently large number of stimulus trials and conditions.

The independence between the P_2 modulation and the ongoing EMG pattern was seen in all flexors studied. Moreover, when different flexors were compared it was found that their P_1 and P_2 responses occurred at the same latency and that the modulation of the P_2 responses during the step cycle was very similar despite large differences in anatomical location and in activity patterns among these muscles during normal walking. When P_2 responses were small or irregular, they still typically clustered around the time of foot lift at end stance (Fig. 6). The P_1 and P_2 responses usually appeared in combination, but there were some examples of either one appearing without the other (Fig. 7). The P_2 responses disappeared during sleep while P_1 responses remained unaffected. Increases in stimulus intensity resulted in decreases in minimal latencies of P_1 and P_2 responses (Table 1, based on averaged responses during walk-

ing). In contrast, the latency of the P_3 responses increased at higher intensities. The latter was presumably due to an increase in the inhibition preceding P_3 responses.

At high stimulus intensities, the integrated reflex amplitudes could reach up to 10 times the control EMG values. Under these conditions one could find P_2 activity at end stance and early swing in some extensors such as SOL, SM, and FDL, which are in close proximity to flexor muscles, but not in the more superficial extensors such as QA and MG (see Table 2). Since it is likely that this P_2 activity is at least partly due to EMG cross talk, it will not be described here. For lower levels of reflex EMG amplitude, the possibility of cross talk is less likely since the normal EMG traces of extensors showed no activity during swing and since flexors were silent during stance (Fig. 1). The EMG of FDL presented some difficulty in that two bursts were seen: a low-amplitude long-lasting burst throughout early stance and, irregularly, a second short large-amplitude burst at end stance. In view of its proximity to the pretibial flexor muscles, it is not certain how much of this activity was due to pickup. From preliminary data (M. J. O'Donovan, personal communication), it appears that the long-lasting extensor burst (seen by us and others) persists after cutting the nerve to FDL. In view of this uncertainty, the data on FDL will be mentioned only in passing. At high stimulus intensities (20 mA), a significant P_2 response was detected in MG following ankle stimulation even during early swing (Table 2). This response could not have

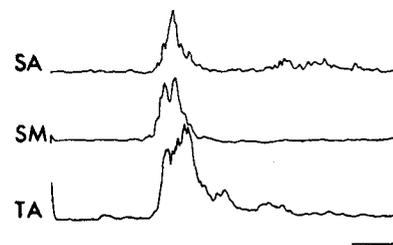


FIG. 7. Near-complete absence of P_1 responses (10 ms) despite large P_2 responses (25 ms) following stimulation of the dorsum of the foot with single pulses of 10 mA in the resting animal. Averages of eight rectified and integrated EMG responses. Time calibration: 10 ms.

been due to cross talk (SOL being silent) but was presumably due to maximal activation of a specific MG reflex pathway (see DISCUSSION). On the other hand, significant P_2 flexor responses were still found during early stance (Table 2) although such responses were generally suppressed during this period. This again emphasizes that activity in reflex pathways was modulated and not switched on and off.

STIMULUS LOCATION. Stimulation of the dorsum of the foot yielded essentially the same results as when ankle stimuli were used (Fig. 5 and Table 2). The P_2 responses at end stance appeared more readily in ST following stimulation of the dorsum of the foot, but this was only a relative difference. The only consistent fundamental difference was the absence of MG activation at P_2 latency during the early stance phase (Figs. 5 and 8). With strong stimulation of the dorsum of the foot, a small amount of MG activation was sometimes seen, but this may have been due to current spread to the neighboring skin of the ankle zone (Fig. 5). Normally, the reflex activation of MG following stimulation of any skin site, other than the lateral surface of the ankle, occurred at the same latency as the P_3 in other extensors (Fig. 8). The only extensor that never showed a P_3 response during early stance was SOL, although this muscle is a close synergist of MG. Hence, the P_2 activation of MG during early stance seemed to be a reflex action reserved to afferents from the skin on the lateral surface of the ankle, where the application of 1-mA stimuli was sufficient for its evocation. It was not de-

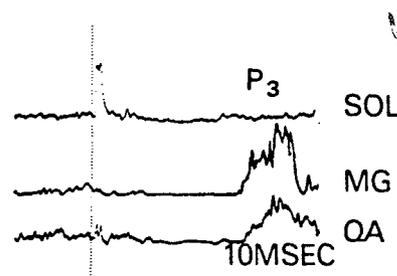


FIG. 8. Averaged rectified reflex responses in three extensor muscles (SOL, MG, and QA) following single 1-ms pulses of 20 mA applied to the dorsum of the foot (vertical dotted line) only during a 200-ms period following foot placing ($n = 8$).

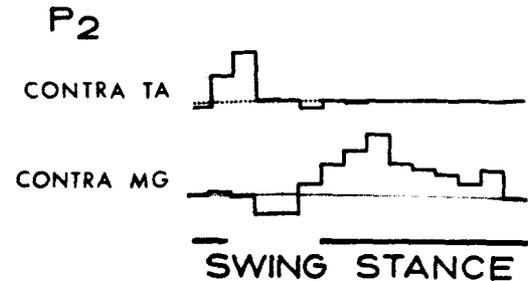


FIG. 9. Modulation of P_2 reflex responses in the contralateral TA and MG following 8.5-mA stimulation of the skin of the lateral surface of the ankle. The peak amplitudes of the P_2 responses were, on the average, 680 and 300% of the control EMG amplitudes, respectively.

termined whether many extensors other than MG participated in the P_2 activation during early stance, but in a few cases it was noted that FDL showed similar responses.

Another example of local sign reflex action was found when stimuli were applied to the plantar surface of the foot. In a few cases it was found that such stimuli did not yield the usual P_1 , P_2 activation in flexors, but instead gave P_1 responses followed by a 20-ms period of inhibition. An example of such flexor inhibition is given in Fig. 1, which was composed from SA responses to 20-mA plantar stimuli.

Contralateral responses

In the unstimulated limb, the responses consisted of a single peak at a latency of 20-25 ms in flexors and extensors. The modulation of the amplitude of these responses was essentially the same as the modulation of the ipsilateral P_2 responses in flexors and the P_3 responses in extensors (Fig. 9). Crossed responses appeared in TA around the period of foot lift while crossed P_2 responses occurred in MG during stance. Since only two muscles (TA and MG) were recorded from in the hindlimb contralateral to the skin electrodes, it was not possible to establish in detail the distribution of the crossed reflex effects in a representative sample of muscles following skin stimulation. However, it was found that most reflex effects could be reproduced when certain muscles such as FDL or MG were stimulated in the same manner as the skin, pre-

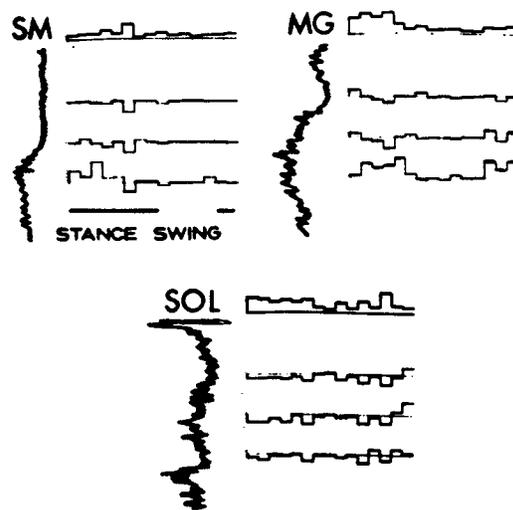


FIG. 10. Modulation of contralateral reflex responses in SOL, MG, and SM of the left hindlimb following stimulation at 20 mA (1 ms) through intramuscular electrodes implanted in MG of the right hindlimb. Note the absence of a crossed response at P_2 latency in SOL.

sumably through activation of nearby cutaneous and/or muscle afferents. Hence, in a few experiments, the right MG was stimulated with 20-mA stimuli and the distribution of the reflex effects was studied in the crossed (left) hindlimb. It was found that those muscles that responded as flexors ipsilaterally did so contralaterally as well and thus showed maximum reflex activity at end stance for that limb. Similarly, the same extensors that showed a late P_3 activation following ipsilateral stimulation during stance exhibited contralateral P_2 responses as well (Fig. 10, SM and MG). SOL, which always lacked an excitatory response ipsilaterally, failed to show any response contralaterally as well (Fig. 10, SOL).

DISCUSSION

The present finding that weak electrical stimulation of various skin areas of the hindlimb leads to short- and long-latency responses (referred to as P_1 and P_2 at 10 and 25 ms) in flexors when given at the start of the swing phase confirms the results of Forssberg (13) who introduced the term "stumbling corrective reaction" to describe

such responses. In addition, by recording simultaneously from up to 10 different muscles, it was possible to extend Forssberg's observations to muscles from different articulations and from both hindlimbs. It was found that different hindlimb flexors such as SA, ST, TA, and EDL were very similarly recruited in P_1 and P_2 responses and that the P_2 responses always showed a maximum near the transition from stance to swing. The similarity of the modulation of the reflex responses of all flexors during the step cycle was surprising in view of the differences in EMG activity pattern that these muscles exhibited during walking. For most flexor muscles, there was a clear discrepancy between the normal EMG and reflex response patterns. Similar discrepancies were already noted by Forssberg (13), but only for ST.

Two mechanisms may be involved: 1) All flexor motoneurons may receive a phase-advanced subliminal drive, for example from the flexor part of a hypothetical locomotor oscillator. The presently described reflex modulation then reflects the time course of this subthreshold activation. If this hypothesis were valid, one would expect that P_1 and P_2 responses would be very similarly gated since only the depolarization level of the motoneurons would be important. This was not always the case, however, and in both Forssberg's study (13) and the present one, it was found that P_1 responses could occur without concomitant P_2 responses, and vice versa. 2) A second possibility involves premotoneuronal "gating" of reflex pathways, perhaps through the intervention of a common flexor center controlling all hindlimb flexors synchronously (see Refs. 14, 19). Such a center should be situated at the spinal cord level since spinal cats show "phasic gain control" (14). Evidence for such a "flexor center" is still limited (5, 7, 16), but the idea that at least part of the flexor reflex spinal circuitry belongs to the flexor part of the central locomotor oscillator has a very long tradition, originating from the work of Sherrington (23).

The gradual increase in the amplitude of the P_2 flexor responses during the last part of stance would be explained as a slow disinhibition of a locomotor flexor center,

which would reach a maximum of activity just before the onset of the swing phase (see Refs. 6, 25). In view of the low stimulus intensity needed to elicit flexor responses during the period surrounding foot lift, it seems quite plausible that under normal conditions the afferent activity from various skin regions could contribute to the initiation of the flexion phase at end stance. Later in the swing phase there is a substantial decrease in the amplitude of the flexor responses, although many flexors remain normally active during this period. The observation that reflex activation of flexors is much more easily obtained at the start of the flexor EMG burst period during walking agrees well with previous data from premammillary cats, where it was found that flexor reflex excitation occurs only for certain stimuli given at the beginning of the flexor activity during walking, while later stimuli induced flexor inhibition instead (10). Flexor inhibition, such as found in the latter study, was observed here as well, namely, following stimulation of the plantar surface of the foot. However, the inhibition was not strong enough to cause a phase reversal as was observed in the fixed hindlimb of walking premammillary cat (10).

Extensor inhibition and rebound

Lifting of the foot would be a very inappropriate reflex response for a hindlimb that is in the middle of its load-bearing period during stance. Both in this study and in previous ones (12, 13, 28), it was found that stimuli given during this period did indeed fail to produce much flexor excitation unless very strong stimuli were used. Instead, an overall extensor inhibition was seen, followed by a later recovery at 35 ms (P_3). The P_3 responses in extensors differed fundamentally from P_2 flexor responses in that they 1) had a longer latency, 2) always followed inhibition, and 3) had an amplitude that closely followed the degree of activation of the muscle during walking. These results agree with Forssbergs' (13) data, but he also described late excitatory responses in LG at the end of the flexor period. From this he concluded that postinhibitory rebound is not a likely explanation for these longer latency responses. In the present study it was shown that gastrocnemius

reflex excitation can indeed occur during swing (Table 2), presumably through activation of a specialized pathway (see further). However, typical P_3 responses in various extensors were only seen during stance after a period of inhibition. Hence, we feel that the present data support the notion of some postinhibitory rebound of mechanical or neuronal origin. Spindle stretching and loss of Golgi tendon organ inhibition in the yielding of inhibited extensors could have provided reflex excitation, but the presence of P_3 responses during the first extension phase, before foot placing (seen also by us, Fig. 4) do suggest that proprioceptive activity is not likely to be the sole source of excitation. P_3 responses may also have resulted from direct reflex excitation due to the skin stimulation. Cutaneous extensor reflexes involving many hindlimb extensors have been described (for references see Ref. 11). Involvement of long-loop reflexes cannot have been very important since a similar inhibition with ensuing rebound was found in spinal cats (22) and spinal man as well (15).

Since P_3 extensor responses differ in latency from P_2 flexor responses and can occur simultaneously at some phases of the step cycle and since P_2 flexor and extensor responses can occur in the "wrong" phase (Table 2), it does not seem appropriate to use the term "phase-dependent reflex reversal" to describe the present results. Rather, the data are consistent with the modulation of a dominant single system providing P_2 excitation in flexors and P_2 inhibition in extensors. In addition, however, some extensors seem to receive reflex excitation from a parallel system. The pattern of reflex responses during stepping easily separated flexors from extensors. However, the bifunctional SM showed a typical flexor P_1 excitation in early swing as well as an extensor-type inhibition and rebound in early stance. Our data do not distinguish whether these opposite effects were due to selective activity of different parts of the muscle, but recent data would support such a hypothesis (4, 26).

Soleus and gastrocnemius

Following stimulation of the skin innervated by the sural nerve during early

stance, a large P_2 - P_3 reflex activation of MG was seen, as could have been expected on the basis of previous results using direct stimulation of the sural nerve in premammillary or intact walking cats (11, 12). In contrast, SOL only showed inhibition during this period. Similarly, in immobile decerebrate cats, natural or electrical stimulation of the sural nerve produced excitation preferentially in large motor units, well represented in MG, and inhibition preferentially in small motor units, of which SOL is exclusively comprised (8, 17). In the present study, the differential reflex control of MG and SOL proved to be more generalized since SOL also failed to participate in other extensor responses such as the P_3 rebound following stimulation of the dorsum of the foot or the crossed P_2 extensor response. It should be noted, however, that the failure to detect any excitatory peak does not exclude the possibility of an excitatory drive to SOL since the more pronounced inhibition of this muscle may mask all traces of excitation.

The total absence of visible SOL reflex activation in the present context is in sharp contrast to the finding that Ia EPSPs are largest in slow motor units (9). This suggests that SOL, a slow muscle that contributes mostly to triceps surae force during quiet standing and slow walk (27), would be most useful at responding to purely proprioceptive perturbations, while the faster MG is used specifically for fast movements (such as paw shakes, see Ref. 24) in response to cutaneous (FRA mediated) events.

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REFERENCES

- ANDERSSON, O., FORSSBERG, H., GRILLNER, S., AND LINDQVIST, M. Phasic gain control of the transmission in cutaneous reflex pathways to motoneurons during "fictive locomotion." *Brain Res.* 149: 503-507, 1978.
- BAK, M. J. AND LOEB, G. E. A pulsed integrator for EMG analysis. *Electroencephalogr. Clin. Neurophysiol.* 47: 738-741, 1979.
- BAK, M. J. AND SCHMIDT, E. M. An analog delay circuit for on-line visual confirmation of discriminated neuroelectric signals. *IEEE Trans. Biomed. Eng.* 24: 69-71, 1976.
- BAWA, P. AND TATTON, W. G. Motor unit responses in muscles stretched by imposed displacements of the monkey wrist. *Exp. Brain Res.* 37: 417-438, 1979.
- BAYEV, K. V. Central locomotor program for the cat's hindlimb. *Neuroscience* 3: 1081-1092, 1978.
- BERKINBLIT, M. B., DELIAGINA, T. G., FELDMAN, A. G., GELFAND, I. M., AND ORLOVSKY, G. N. Generation of scratching. I. Activity of spinal interneurons. *J. Neurophysiol.* 41: 1040-1057, 1978.
- BROWN, T. G. On the nature of the fundamental activity of the nervous centres: together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. *J. Physiol. London* 48: 18-46, 1914.
- BURKE, R. E., JANKOWSKA, E., AND TEN BRUGGENCATE, G. A comparison of peripheral and rubrospinal synaptic input to slow and fast twitch motor units of triceps surae. *J. Physiol. London* 207: 709-732, 1970.
- BURKE, R. E., RYMER, W. Z., AND WALSH, J. V. JR. Relative strength of synaptic input from short-latency pathways to motor units of defined types in cat medial gastrocnemius. *J. Neurophysiol.* 39: 447-458, 1976.
- DUYSSENS, J. Reflex control of locomotion as revealed by stimulation of cutaneous afferents in spontaneously walking premammillary cats. *J. Neurophysiol.* 40: 737-751, 1977.
- DUYSSENS, J. AND PEARSON, K. G. The role of cutaneous afferents from the distal hindlimb in the regulation of the step cycle of thalamic cats. *Exp. Brain Res.* 24: 245-255, 1976.
- DUYSSENS, J. AND STEIN, R. B. Reflexes induced by nerve stimulation in walking cats with implanted cuff electrodes. *Exp. Brain Res.* 32: 213-224, 1978.
- FORSSBERG, H. Stumbling corrective reaction: a phase-dependent compensatory reaction during locomotion. *J. Neurophysiol.* 42: 936-953, 1979.
- FORSSBERG, H., GRILLNER, S., AND ROSSIGNOL, S. Phasic gain control of reflexes from the dorsum of the paw during spinal locomotion. *Brain Res.* 132: 121-139, 1977.
- HUGON, M. *Reflexes polysynaptiques cutanes et commande volontaire* (these). Paris, 1967.
- JANKOWSKA, E., JUKES, M. G., LUND, S., AND LUNDBERG, A. The effect of dopa on the spinal cord. 5. Reciprocal organization of pathways

- transmitting excitatory action to alpha motoneurons of flexors and extensors. *Acta Physiol. Scand.* 70: 369-388, 1967.
17. KANDA, K., BURKE, R. E., AND WALMSLEY, B. Differential control of fast and slow twitch motor units in the decerebrate cat. *Exp. Brain Res.* 29: 57-74, 1977.
 18. LOEB, G. E. AND DUYSSENS, J. Activity patterns in individual hindlimb primary and secondary spindle afferents during normal movements in unrestrained cats. *J. Neurophysiol.* 42: 420-440, 1979.
 19. PERRET, C. AND CABELGUEN, J.-M. Main characteristics of the hindlimb locomotor cycle in the decorticate cat with special reference to bifunctional muscles. *Brain Res.* 187: 333-352, 1980.
 20. PROCHAZKA, A., SONTAG, K.-H., AND WAND, P. Motor reactions to perturbations of gait: proprioceptive and somesthetic involvement. *Neurosci. Lett.* 7: 35-39, 1978.
 21. SHERRINGTON, C. S. *The Integrative Action of the Nervous System.* New Haven: Yale University Press, 1906.
 22. SHERRINGTON, C. S. On reciprocal innervation of antagonistic muscles. Eleventh Note.—Further observations on successive induction. *Proc. R. Soc. London Ser. B* 80: 53-71, 1908.
 23. SHERRINGTON, C. S. Flexion-reflex of the limb, crossed extension reflex, and reflex stepping and standing. *J. Physiol. London* 40: 28-121, 1910.
 24. SMITH, J. L., BETTS, B., EDGERTON, V. R., AND ZERNICKE, R. F. Rapid ankle extension during paw shakes: selective recruitment of fast ankle extensors. *J. Neurophysiol.* 43: 612-620, 1980.
 25. VIALA, D. Donnees sur l'activation due generateur spinal de la locomotion par les voies noradrenergiques et par les afferences due reflexe de flexion. *J. Physiol. Paris* 72: 31A-32A, 1976.
 26. VIDAL, C., VIALA, D., AND BUSER, P. Central locomotor programming in the rabbit. *Brain Res.* 168: 57-73, 1979.
 27. WALMSLEY, B., HODGSON, J. A., AND BURKE, R. E. Forces produced by medial gastrocnemius and soleus muscles during locomotion in freely moving cats. *J. Neurophysiol.* 41: 1203-1216, 1978.
 28. WAND, P., PROCHAZKA, A., AND SONTAG, K.-H. Neuromuscular responses to gait perturbations in freely moving cats. *Exp. Brain Res.* 38: 109-114, 1980.