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Neural Control of Limb Coordination. I. Comparison of Hatching and Walking Motor Output Patterns in Normal and Deafferented Chicks

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Previous work has shown that the neural circuits underlying the leg movements of walking and hatching coexist in post-hatching chicks (Bekoff and Kauer, 1984). In the present study, quantitative analysis of leg EMGs shows that there are some similarities, but also significant differences, in the motor output patterns of walking and hatching. This study examines the effect of removing sensory feedback from the legs on the production of the distinctive leg motor patterns. The temporal characteristics and interlimb coordination of hatching and walking are little affected. However, major changes in intralimb motor output patterns are seen when compared to records from normal chicks. These changes fall into one of 2 categories. Some parameters show similar changes in both behaviors after deafferentation (e.g., increases in flexor burst durations and cycle period). This suggests that certain features of sensory input from the legs normally modulate the hatching and walking pattern-generating circuitry in similar ways. Other parameters show convergence. That is, these aspects of the 2 intralimb motor patterns become more similar to each other after removal of sensory input. This is consistent with the hypothesis that some feature of sensory input from the legs normally modulates one set of multiuse intralimb circuitry to produce different output patterns. In general, the walking pattern becomes more like hatching after deafferentation, rather than the reverse, which suggests that the hatching pattern is a more basic one. The maintenance of some residual differences in intralimb motor patterns after leg deafferentation suggests that other sources of modulation must also be involved, or that there are some additional elements of circuitry that are called into play during the normal production of walking and hatching.

Many different behaviors in any limbed animal's repertoire involve coordinated limb movements. In a bird, for example, the legs participate in behaviors as diverse as embryonic motility, hatching, walking, and courtship displays. It is clear that production of the distinctive leg movements used in these different behaviors involves activation of the same set of muscles and motor neurons. However, it is not known to what extent the neural pattern-generating circuitry for the different behaviors share the same interneurons. One possibility is that separate pattern-generating circuits could be used to produce the leg movements of each behavior. Alternatively, the same pattern-generating circuitry could be involved in all of the behaviors (Bekoff, 1976, 1978). Finally, it is possible that the production of the limb movements of different behaviors could involve the use of some separate, as well as some common, circuitry.

On the basis of similarities in the motor output patterns, the suggestion has been made that one pattern-generating circuit may be used to produce several different behaviors in invertebrates (Huber, 1962; Kammer, 1968, 1970; Wilson, 1968; Elsner, 1974; Ayers and Davis, 1977; Ayers and Clarac, 1978; Pfluger and Burrows, 1978; Simmers and Bush, 1983). However, the underlying cellular mechanisms have not been examined in these systems. Evidence for several mechanisms for modulating the output of a basic, multiuse pattern-generating circuit has been presented. These include modulation by sensory input (Reingold and Camhi, 1977; Sherman et al., 1977), descending input (Mesce and Truman, 1985, 1986), neurotransmitters (Marder and Hooper, 1985) and other synaptic interactions (Getting, 1987).

It has also been proposed that multiuse pattern-generating circuits are present in vertebrates (see Grillner, 1981, for a review). For example, Berkinblit and colleagues (1978a, b) suggest that scratching and walking are produced by the same intralimb circuitry in cats. In addition, analyses of walking, airstepping and paw-shake responses in spinal cats, 3 different forms of the scratch reflex in spinal turtles, and undulatory behaviors in lamprey also provide suggestive data on the use of the same intralimb neural pattern-generating circuitry (Ayers et al., 1983; Giuliani and Smith, 1985; Robertson et al., 1985; Smith et al., 1986). Although this is a reasonable hypothesis for vertebrates, there is as yet no conclusive evidence for the use of the same neural pattern-generating circuitry to produce different behaviors, either in adults or at different stages during ontogeny.

The production of the leg movements of walking and hatching in chicks provides an ideal system for an analysis of this issue. A great deal of work has already been done on the neural circuitry underlying walking in a variety of other vertebrates, including chicks (Landmesser, 1978; Grillner, 1981; Jacobson and Hollyday, 1982a, b). This work shows that there is a spinal central pattern-generating circuit for each limb. This *intralimb* circuit generates the basic locomotor pattern for a single limb.

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To produce the various *interlimb* coordination patterns associated with different gaits, the individual intralimb circuits are coordinated with one another in different patterns (Forssberg et al., 1980; Halbertsma, 1983; Smith et al., 1986).

The leg motor output patterns involved in hatching in chicks have also been characterized (Bekoff, 1976; Bekoff and Kauer, 1982, 1984). Furthermore, the pattern-generating circuitry for the leg movements of hatching is still present and can be turned on in hatchling and young adult chickens if they are folded into the hatching position and placed in artificial glass eggs (Bekoff and Kauer, 1984). The specific stimulus is the tight bending of the neck to the right or left (Bekoff and Kauer, 1982). These studies show that the pattern-generating circuitry for the leg movements of both hatching and walking coexist in the post-hatching chick.

The present study addresses the issue of whether the production of the distinctive leg movements of hatching and walking involves the use of the same, or separate, intralimb pattern-generating circuitry. To examine this issue, we first quantify the differences between hatching and walking leg motor output patterns. If the same circuitry is used, then one source of the differences in motor output patterns could be modulation due to sensory input from the legs. A chick receives different sensory feedback depending on whether it is hatching or walking. During hatching, the legs are tightly flexed and can extend only slightly before they are stopped by the shell. On the other hand, during walking, the legs are in a more extended position and the chick must support its weight against gravity. Therefore, in the second part of this study, we analyze the effects of removing sensory feedback from the legs on walking and hatching motor output patterns. A preliminary account of some of the results has appeared (Bekoff, 1982).

Materials and Methods

Animals

Fertile eggs from Grey Leghorn and Shaver strain chickens were obtained from Great Western Poultry (Greeley, CO) and were incubated under standard conditions. The day of hatching is called posthatching day 0. After hatching, chicks were maintained in groups in heated chambers with food and water ad libitum. Because no differences were found in the results obtained from the 2 strains of chickens, they have been combined.

Deafferentation

Zero- to 2-day-old posthatching chicks were anesthetized with halothane. The lumbosacral dorsal roots were exposed by laminectomy. The 8 roots that contribute to hindlimb innervation, L1 to S3, were cut bilaterally with sharpened forceps. Prior to being used in an experiment, each chick was tested for success of the hindlimb deafferentation procedure by pinching the limb with forceps in a variety of locations. If responses to sensory stimulation were obtained, the chick was not used in this study. In addition, after completion of an experiment, the success of the deafferentation procedure was confirmed by visual inspection of the cut dorsal roots. If any of the roots were partially or completely intact, the data from that chick were discarded.

Note that this deafferentation procedure eliminates sensory input from the legs. However, the non-limb innervating sacral roots, those posterior to S3, were left intact, as were all roots anterior to the lumbosacral region. This allowed afferent input from the tail and wings to be used to turn on walking, and afferent input from the neck to be used to turn on hatching (see Experimental conditions).

EMG implantation

Normal or deafferented 0–2-d-old posthatching chicks were anesthetized with halothane. Bipolar hook electrodes made from Teflon-coated stainless steel wire (100 μm diameter) were implanted in 4 leg muscles as described previously (Bekoff and Kauer, 1984). The wires were attached to long, flexible leads so that the chicks could move freely. At the completion of an experiment, the precise location of each electrode was determined by dissection and visual inspection.

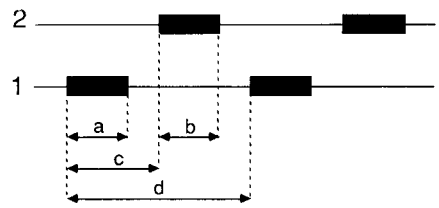

Figure 1. Parameters used in analyzing EMG records. *a,* Muscle *1* burst duration; *b,* muscle *2* burst duration; *c,* latency of onset of muscle 2 with respect to muscle 1; *d,* cycle period. Phase of muscle 2 with respect to muscle 1 was calculated as *c/d.* Right GL was always used as the reference trace, muscle 1.

In these experiments, 6 different muscles were studied extensively. These included 3 pairs of muscles whose major actions are antagonistic at hip, knee, or ankle. These muscles (abbreviation and alternative names given in parentheses) and their actions are: caudilioflexorius (CF; semitendinosus), a hip extensor and weak knee flexor; sartorius (SA), a hip flexor and knee extensor; femorotibialis (FT; quadriceps femoris), a knee extensor; iliofibularis (IF; biceps femoris), a knee flexor and hip extensor; gastrocnemius lateralis (GL), an ankle extensor; and tibialis anterior (TA), an ankle flexor. See Bekoff and Kauer (1984) and Jacobson and Hollyday (1982a) for further descriptions of these muscles.

In each experiment, the right and left GL and 2 other muscles of the right leg were implanted. The right and left GL were used to monitor interlimb motor output patterns. The right GL and the 2 other right leg muscles were used to monitor intralimb motor output patterns.

Experimental conditions

Hatching. Normal or deafferented chicks were gently folded into the hatching position with the head bent to the right (Hamburger and Oppenheim, 1967). Each chick was then placed in an artificial glass egg (Bekoff and Kauer, 1984). The egg was placed horizontally on a plastic egg holder and temperature was maintained at about 34°C with a lamp. Hatching typically began within 2 min under these conditions. Previous work has shown that afferent input resulting from bending the neck into the hatching position is a selective trigger for turning on the hatching motor output (Bekoff and Kauer, 1982). The leg motor output pattern seen during this "glass egg hatching" does not differ from that of normal hatching in any of the parameters being examined in this study (Bekoff and Kauer, 1984).

Walking. Walking was elicited from normal chicks in 2 ways. In the first, the chicks walked freely on a straight, stationary runway toward a light. In the second, the chicks were suspended in a sling with their feet on a treadmill moving at 0.15–0.25 m/sec. The movement of the treadmill was sufficient to elicit walking in these chicks.

Because chicks with deafferented legs are unable to balance themselves, free walking was not obtained. The deafferented chicks were suspended in a sling with their feet on the moving treadmill, and walking was elicited by gently pinching the wings and/or tail.

Data collection and analysis

EMG recordings were made from normal and deafferented chicks during both hatching and walking. The records were taped on a TEAC 3340s tape recorder for later filming and analysis.

Filmed EMG records were digitized as shown in Figure 1. Muscle burst duration, latency, cycle period, and phase were calculated. To analyze interlimb coordination, phase relationships of left GL with respect to right GL were calculated. To analyze intralimb coordination, phase relationships of various right leg muscles with respect to right GL were calculated. If a muscle showed 2 bursts of activity per cycle period, the 2 bursts were designated "A" and "B" and were analyzed separately. For the purposes of this study, EMG bursts will be classified as extensor or flexor bursts, depending on whether they are active during GL (ankle extensor) bursts, and thus are part of an extensor synergy, or are active during TA (ankle flexor) bursts and are therefore part of a flexor synergy. Note that this classification is not based on information about whether the muscle activity results in flexion or extension. Nevertheless, in most

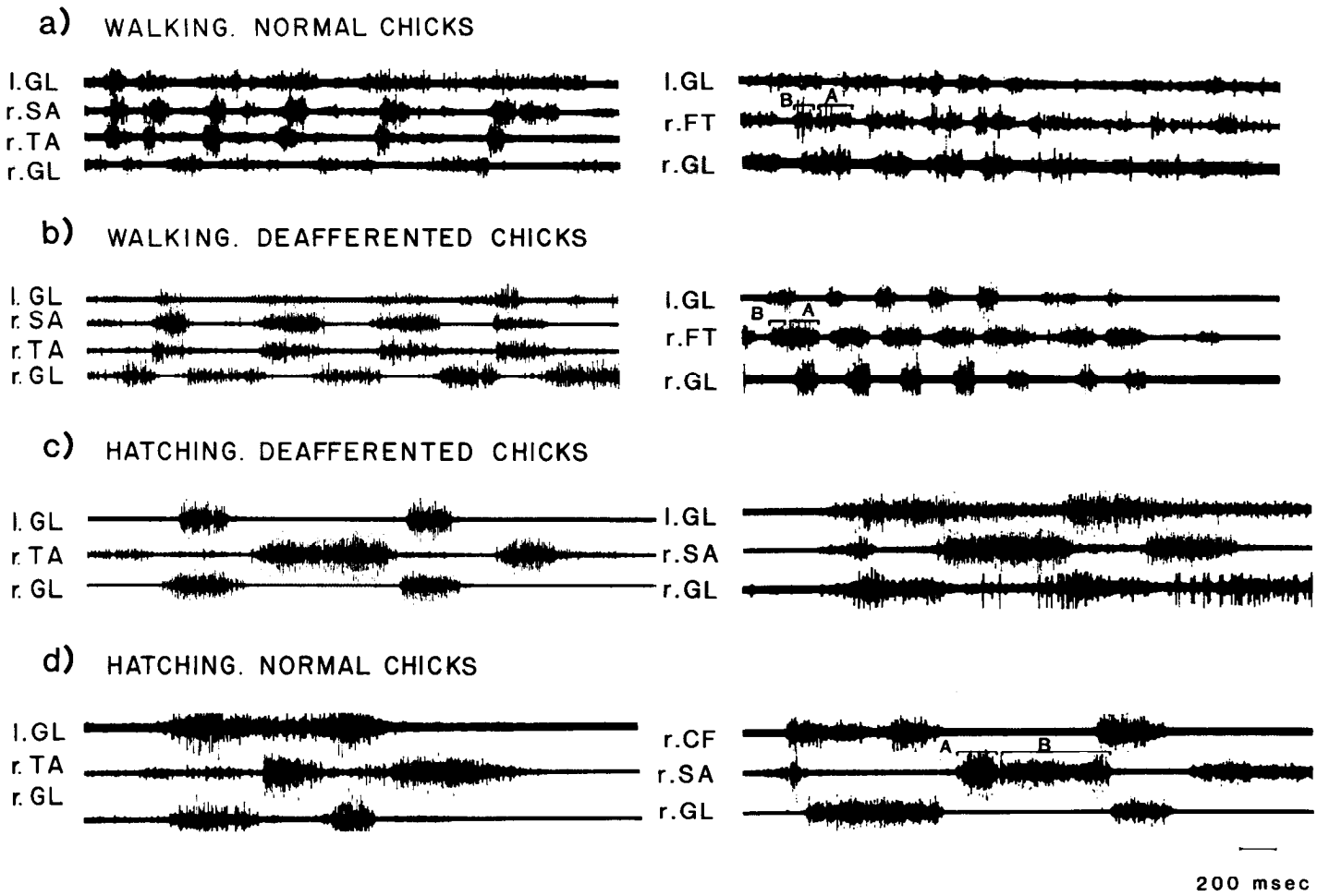

Figure 2. EMG records from walking and hatching in normal and deafferented chicks. An A and B mark the 2 bursts of double-bursting muscles. FT shows 2 bursts during normal and deafferented walking and SA shows 2 bursts during normal hatching.

cases the classification accurately reflects the function of the burst as determined for walking by Jacobson and Hollyday (1982a). The one exception is the FT_b burst of FT, which, according to this scheme, would be classified as both a "flexor" and an "extensor" burst, although this muscle is a pure knee extensor (Jacobson and Hollyday, 1982a).

One-way analysis of variance was used to determine whether there were statistically significant ($p < 0.05$) differences among mean values. The Scheffé a posteriori test was used to determine where the significant differences occurred. Regression analyses were used to compare relationships between parameters (e.g., burst duration and cycle period; burst duration and latency). Pearson's product–moment correlation coefficients (r's) were calculated for the regressions. The significance of differences between regressions was determined using the method presented in Zar (1974).

Results

Characterization of normal hatching and walking motor output patterns

The first step in this study was to compare the leg motor output patterns characterizing hatching and walking, in order to identify and quantify the parameters that distinguish them. No significant differences were found in any of the parameters analyzed when the motor output patterns seen during walking on a runway and walking on a moving treadmill were compared (see also Jacobson and Hollyday, 1982a). The data presented below for normal walking are from chicks walking on a runway.

EMG recordings from leg muscles reveal striking differences between the leg motor output patterns typical of hatching and walking (Fig. 2, *a, d*). First, they differ in temporal organization. Walking is a cyclically repetitive behavior in which sequences of highly variable length can occur (Fig. 2*a*). In contrast, hatching is an episodic behavior; each episode lasts 1–3 sec. Within an episode, the leg motor output consists of 1–3 extension–flexion sequences (Fig. 2*d*; see also Bekoff, 1976; Bekoff and Kauer, 1984). Hatching episodes alternate with periods of inactivity lasting about 20 sec on average (Hamburger and Oppenheim, 1967; Kovach, 1970; Bakhuis, 1974; Bekoff and Kauer, 1984).

Interlimb coordination patterns are also distinctive. Walking involves alternating stepping movements, while hatching involves synchronous extensions and flexions of the legs (Fig. 2, *a, d*). Results of quantitative analysis of interlimb coordination patterns are shown in Table 1. For walking, the mean phase of left GL with respect to right GL is near 0.50, indicating a pattern of alternation. For hatching, a mean value of 0.00 is found for phase of left GL with respect to right GL, indicating a pattern of synchrony.

Quantitative analysis of intralimb motor output shows that there are both similarities and differences in intralimb coordination patterns. For example, muscle pairs tend to show the same general pattern in both behaviors. That is, muscles showing a pattern of alternation during hatching (e.g., GL and TA)

Table 1. Interlimb coordination patterns

Normal walking	Deafferented walking	Deafferented hatching	Normal hatching
0.45 ± 0.00[a]	0.48 ± 0.01	0.03 ± 0.01	0.00 ± 0.01
(388)	(191)	(178)	(75)

Phase values were calculated for the left GL with respect to the right GL. Values near 0.50 indicate a pattern of alternation. Values near 0.00 indicate a pattern of synchrony.

[a] Data are means ± SEM. Values in parentheses are sample sizes.

also alternate during walking, while muscles coactive during hatching (e.g., GL and CF) also tend to be coactive during walking (Fig. 3, *a, d*).

On the other hand, there are many differences between the intralimb coordination patterns of hatching and walking. For example, mean burst durations for all muscles examined are longer for hatching than for walking ($p < 0.05$). Mean cycle period length is much longer for hatching (Table 2). The only overlap in cycle period length is seen in the shortest hatching and the longest walking periods: those in the range of 400–600 msec. While the absolute values for burst duration and cycle period are quite different for the 2 behaviors, the variability, as measured by the coefficients of variation calculated for these parameters, are similar.

Another difference is seen when burst duration as a percentage of the cycle period is calculated. During walking, the extensors are active about 60–70% of the cycle period, while flexors are active only 15–35% (Fig. 3*a*). In contrast, during hatching, the bursts are longer, but the percentage of the cycle period during which the extensors and flexors are active (45–60% and 30–45%, respectively) is more similar (Fig. 3*d*).

Also, while the ankle flexor, TA, and the hip flexor, SA, alternate with GL in both behaviors, the mean phase values are signficantly different when walking and hatching are compared (Fig. 3, *a, d*). That is, phase of TA and SA onset is near 0.70 in walking and close to 0.50 in hatching.This remains true even when phase values calculated only from cycle periods in the overlapping part of the range are used. In fact, with the exception of period length, all other differences found between walking and hatching are still seen when only those values taken from cycle periods in the overlapping part of the range are considered.

Two double-bursting muscles show differences in activation pattern when walking and hatching are compared. For example, SA has a distinctive double-bursting pattern during hatching, which is not seen during walking (Figs. 2, *a, d*; 3, *a, d*), while FT has a double-bursting pattern during walking (Figs. 2*a*; 3, *a, d*) that is not seen during hatching.

Another distinctive characteristic of walking that is not shared with hatching is illustrated in Figure 4. In walking, the burst duration of the ankle extensor, GL, shows a marked increase with increasing period length, resulting in a relatively steep slope for the regression (Fig. 4*a*). However, the burst duration of the ankle flexor, TA, increases only slightly, resulting in a significantly shallower slope. Thus, burst durations of extensor and flexor are differentially controlled, with the extensor burst duration being more sensitive to increases in cycle period. In contrast to this, during hatching, both extensor and flexor burst durations increase to a moderate extent as cycle period increases, resulting in slopes of moderate steepness that are not significantly different from one another (Fig. 4*d*). Similar differences

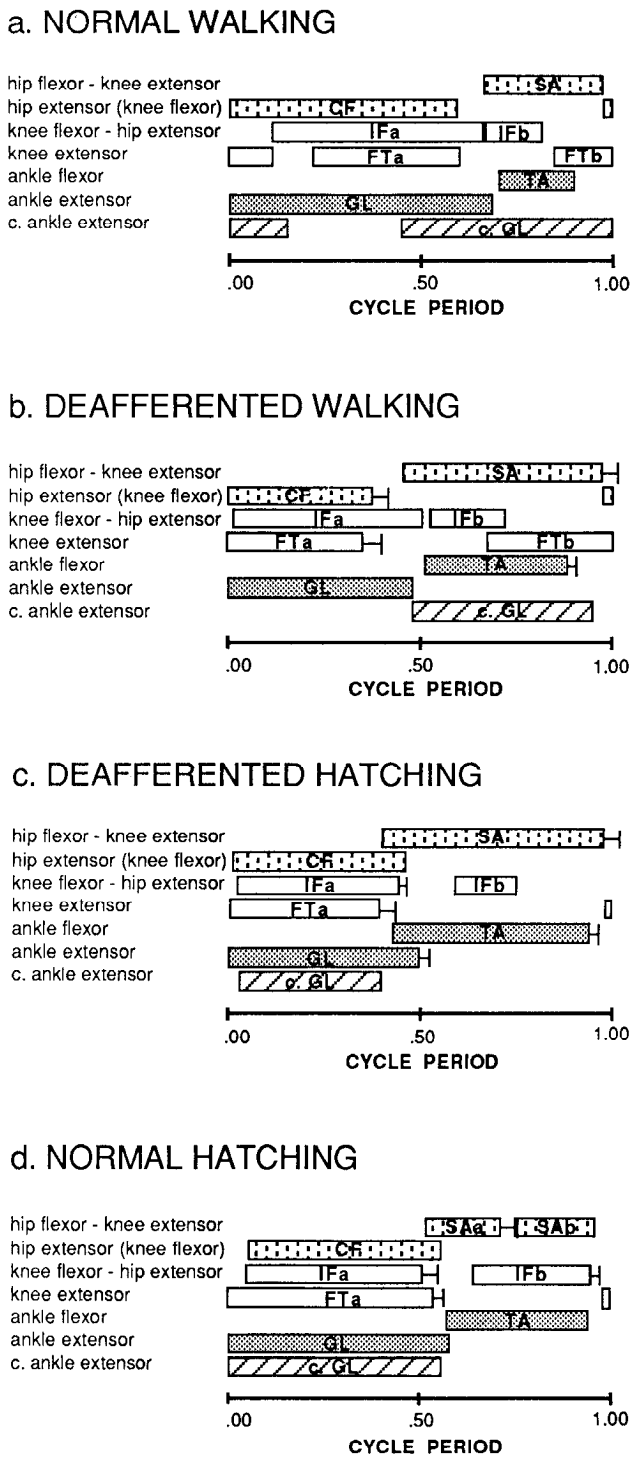

Figure 3. Bar graphs summarizing the results of quantitative analysis of EMG records. Results have been normalized to show one cycle period for each behavior. Abscissa indicates phase of the cycle period. In most cases, *bars* are based on sample sizes of 50 or more cycles. Fewer (20–49) step cycles were used for both bursts of IF and SA in normal hatching and for IF_b in deafferented hatching. SEM for burst durations are indicated by the *narrow lines at the right of bars.* If no error bar is present, the SEM was less than 2% of the cycle period. SEM for phase of onset was 2% or less in all cases and is not shown. Contralateral (*left*) GL is indicated as *c.GL.*

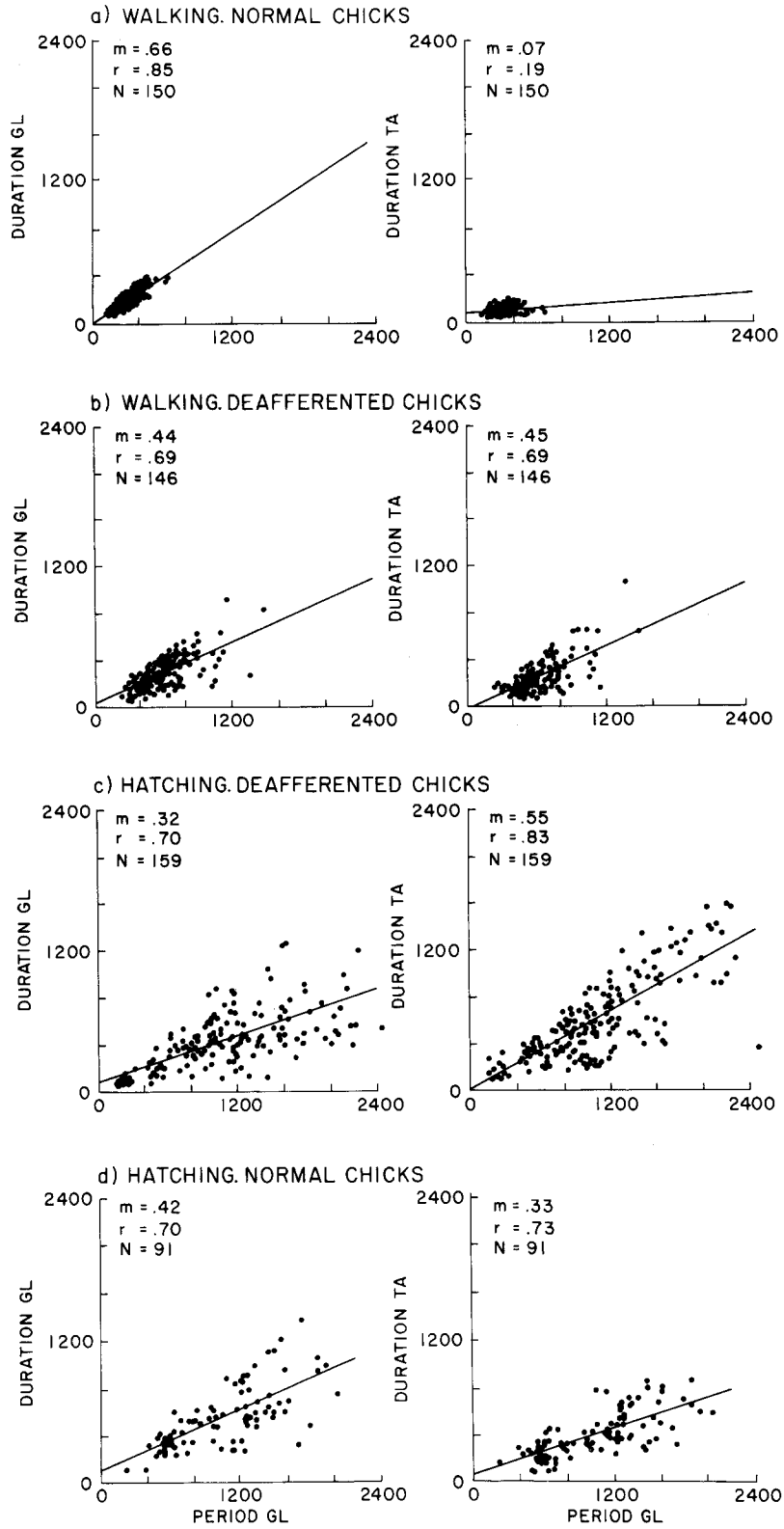

Figure 4. Duration-versus-period graphs. For each of the 4 behaviors, the relationship of the duration of the ankle extensor, GL, and of the ankle flexor, TA, are plotted with respect to period. Each graph in *a, b,* and *c* includes data from ten 0–1-d-old chicks. Each graph in *d* includes data from nine 0–1-d-old chicks. *m* = Slope of the regression. The slope is indicated by the *solid lines on graphs*. *r* = Pearson product–moment correlation coefficient.

between walking and hatching are seen for the hip extensor, CF, and flexor, SA.

Duration-versus-latency relationships of GL and TA also differ for normal walking and hatching (Fig. 5, *a, d*). In both behaviors, the latency of TA with respect to GL is strongly and positively correlated with the duration of GL, with a slope near 1.00 (Fig. 5, *a, d*). That is, TA bursts are initiated near the time that GL bursts terminate. During hatching, the latency of GL with respect to TA is also strongly and positively correlated with the duration of TA, with a slope near 1.00, indicating that GL bursts are initiated near the time that TA bursts terminate. Thus in hatching, the reciprocal relationships are symmetrical. In contrast, during walking, the latency of GL with respect to TA is much less strongly correlated with the duration of TA and

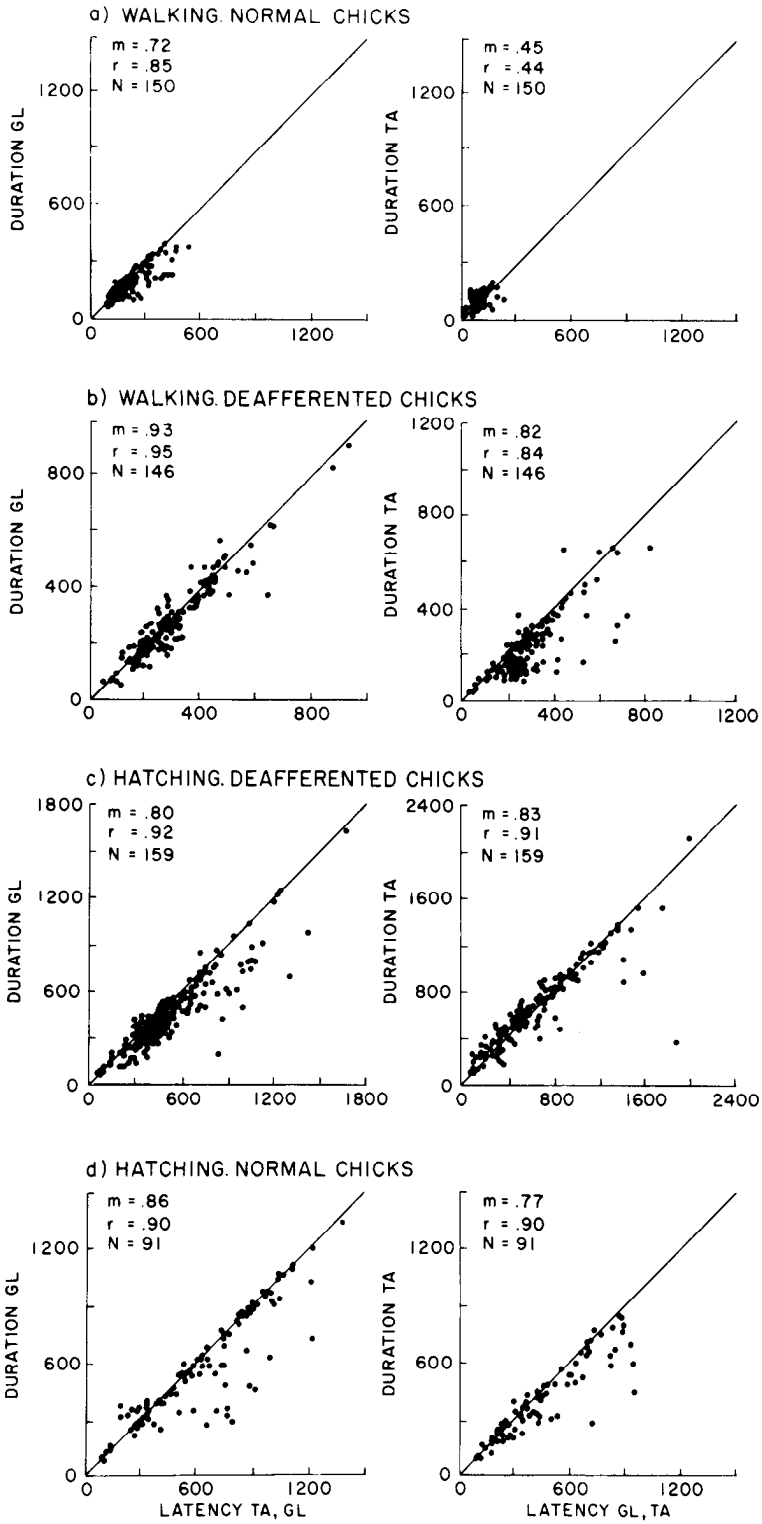

Figure 5. Duration-versus-latency graphs. For the 4 behaviors, the *graphs on the left* show the relationship of the duration of GL to the latency of TA with respect to GL. A 45° *diagonal line* has been drawn on each graph. This line has a slope of 1.00. *Points* that fall on the line indicate cases in which the duration of GL and the latency of TA are equal. That is, in these cases TA bursts turn on just as GL bursts terminate. The same kind of graphs are shown *on the right* for the duration of TA and the latency of GL with respect to TA. Each graph in *a, b,* and *c* includes data from ten 0–1-d-old chicks. Each graph in *d* includes data from nine 0–1-d-old chicks. m = Slope of the regression. r = Pearson product–moment correlation coefficient.

the slope is not near 1.00; therefore, GL bursts are not consistently initiated right at the termination of TA and the reciprocal relationships are asymmetrical.

Comparison of normal and deafferented walking

Some aspects of walking are unchanged after lumbosacral deafferentation. For example, walking is still cyclically repetitive rather than episodic (Fig. 2*b*). Furthermore, the pattern of in-

Table 2. Cycle period

Normal walking	Deafferented walking	Deafferented hatching	Normal hatching
470 ± 9[a]	592 ± 18	1309 ± 33	1270 ± 35
(495)	(150)	(188)	(100)

[a] Data are means ± SEM. Values in parentheses are sample sizes.

Table 3. Percentages of animals showing extensor burst durations that varied with period more than (E > F), the same as (E = F), or less than (E < F) flexor burst durations

	E > F (%)	E = F (%)	E < F (%)
Normal Walking ($N = 21$)	90	5	5
Deafferented Walking ($N = 24$)	38	38	24
Deafferented Hatching ($N = 14$)	14	29	57
Normal Hatching ($N = 15$)	60	33	7

Burst duration of the ankle extensor, GL, was considered to vary with period more than the burst duration of the flexor, TA, if the slope of the regression of duration GL versus period GL was 0.2 units larger than that of duration TA versus period GL. Durations were considered to vary to the same extent if the slopes were within 0.2 units. The burst duration of GL was considered to vary less with period than the burst duration of TA if the slope of the regression of duration GL versus period GL was 0.2 or more units smaller than that of duration TA versus period GL.

terlimb coordination remains one of alternation, as indicated by a mean value near 0.50 for the phase of left GL with respect to right GL (Table 1).

However, a number of features of intralimb coordination change dramatically after deafferentation. For example, flexor burst durations, which are shorter than extensor burst durations during normal walking, become equal to, or even exceed (e.g., for SA), extensor burst durations after deafferentation (Figs. 2, *a, b*; 3, *a, b*). The only significant change in burst duration among extensors is an increase in FT_a. Period length also increases significantly after deafferentation (Table 2). In addition, the variability of burst durations and cycle period, as indicated by the coefficients of variation, increase after deafferentation.

A change is also seen in the percentage of the cycle period during which extensors and flexors are active. A substantial decrease for extensors and an increase for flexors is seen, so that they are all (except IF_b) active during about 40–50% of the cycle period (Fig. 3*b*).

Many phase values are also altered after deafferentation (Fig. 3, *a, b*). For example, the mean phase of the extensor burst of IF, IF_a, also shows a significant decrease, from 0.11 to near 0.01. The phase of FT_a decreases from 0.22 to −0.03. Only the phase of CF remains about the same before (0.02) and after (0.01) deafferentation. Mean phase values of the flexors TA, SA, and the flexor burst of IF, IF_b, decrease significantly, dropping from near 0.70 to 0.50. The mean phase value of the second burst of FT, FT_b, also decreases significantly, from 0.84 to 0.68, but does not approach 0.50. With the exception of FT_b, all the mean values converge toward either 0.50 or 0.00. Note that while phase values for FT_a and FT_b change after deafferentation, the double-bursting pattern, which is a distinguishing characteristic of normal walking, is retained (Fig. 2, *a, b*).

A change is also seen when duration-versus-period relationships are examined. After deafferentation, the slopes of the burst duration-versus-period relationships for extensors (GL or CF) and flexors (TA or SA) have changed (Fig. 4*b*). The slope for the extensors has decreased, while the slope for the flexors has increased. For the ankle muscles, GL and TA, the slopes are now not significantly different from each other. For the hip muscles, CF and SA, the normal relationship is reversed and the flexor slope ($m = 0.50$, $r = 0.73$, $N = 69$) is now significantly steeper than the extensor slope ($m = 0.22$, $r = 0.58$, $N = 88$).

To examine this change in another way, the data from individual chicks were classified into 1 of 3 categories based on the relative steepness of the slopes for the ankle extensor and flexor (Table 3). In over 90% of the chicks in which duration-versus-period relationships were examined during normal walking, the slope for the ankle extensor, GL, was greater than the slope calculated for the ankle flexor, TA. In 5% (one chick), the 2 slopes were similar, and in 5% the relationship was reversed so that the slope of TA was greater than that of GL. In contrast, when deafferented walking was examined, only 38% showed extensor slopes greater than flexor slopes, while an equal percentage showed similar extensor and flexor slopes and 24% showed flexor slopes greater than extensor slopes. Thus, there was much greater variability in this parameter during walking in the deafferented chicks.

After deafferentation, the relationship between the duration of TA and the latency of GL with respect to TA also changes (Fig. 5, *a, b*). That is, after removal of sensory input from the legs, the onset of GL bursts is more strongly correlated with the termination of TA bursts than is the case during normal walking, and the slope increases to near 1.00. Thus the reciprocal duration-versus-latency relationships become symmetrical.

Comparison of normal and deafferented hatching

As in normal chicks, hatching-like behavior typically begins within 2 min after placing deafferented chicks in glass eggs. As compared to hatching in normal chicks, little change is seen in the temporal organization of the bouts of hatching activity; that is, hatching remains episodic (Fig. 2, *c, d*). Furthermore, the pattern of interlimb coordination is not altered from the normal pattern of synchrony. The mean phase of left GL to right GL after deafferentation is still near 0.00 (Table 1).

However, some changes are seen in intralimb motor output. In general, extensor burst durations decrease slightly, although only the change in GL reaches significance. Burst durations of the flexors TA and SA increase after deafferentation; only that of the knee flexor, IF_b, fails to increase significantly. Period length does not change significantly (Table 2). As indicated by the coefficients of variation, the variability of burst durations and cycle period increases after deafferentation whether or not the mean values show significant changes.

In addition to changes in absolute burst duration, extensor bursts decrease slightly to about 40–50% of the cycle period (Fig. 3, *c, d*). Flexor bursts (except IF_b) increase to 50–60%.

The mean phase values for extensor bursts do not change significantly. However, the mean phase values for flexor bursts decrease somewhat after deafferentation and SA activity is no longer divided into discrete A and B bursts (Figs. 2, *c, d*; 3, *c, d*). As during normal hatching, only the FT_a burst of FT is seen and both bursts of IF are retained.

The duration-versus-period relationships change somewhat after deafferentation (Fig. 4, *c, d*). The slopes for ankle extensor and flexor burst durations versus period are both of moderate steepness before and after deafferentation. However, there is a slight decrease in the steepness of the extensor slope and a significant increase in the flexor slope. Similar results are seen for the hip antagonists CF and SA.

Table 3 presents the data for individual chicks, classified into 1 of 3 categories based on relative steepness of the slopes for extensors and flexors. These data show that, during normal hatching, 60% of the chicks show steeper slopes for extensors. In contrast, after deafferentation, nearly the same percentage (57%) show steeper slopes for flexors. Thus, while the slopes

remain moderate in steepness, there is a shift in the distribution across the 3 categories.

After deafferentation, the duration-versus-latency relationships for GL and TA do not change significantly. That is, the reciprocal relationships remain symmetrical (Fig. 5, *c, d*).

Discussion

The analysis of leg motor output patterns of normal hatching and walking reveals that there are substantial differences between the two. They differ in temporal organization in that hatching is episodic, whereas walking is cyclically repetitive. The interlimb coordination pattern of hatching is one of synchrony, while interlimb alternation is seen during walking. Furthermore, while there are some similarities in the intralimb patterns of coordination, clear differences are seen in mean burst durations, cycle periods, phase values, and duration-versus-period and duration-versus-latency relationships. Thus, if the same intralimb pattern-generating circuitry is used to produce both leg motor output patterns, the output of that circuitry must be subject to modulation. In addition, the intralimb circuits must be coordinated with one another to produce the appropriate interlimb patterns and must be activated in the appropriate temporal patterns.

To determine the contribution to these differences made by afferent input from the legs, sensory feedback was removed by bilateral lumbosacral dorsal rhizotomy. While there is evidence that some afferent input travels through ventral roots in cats, this input appears to consist of visceral, and some somatic, nociceptive afferents (Coggeshall, 1980). With one exception, no evidence has yet been produced showing that any known type of natural stimulation results in sensation after dorsal rhizotomy. The exception is that the mechanoreceptive edge cells located in the lateral margins of the spinal cord of lampreys can provide sensory feedback about movement after dorsal rhizotomy (Grillner et al., 1981, 1984). However, the lumbosacral vertebral column of the chick is fused to the synsacrum and is immobile. Therefore, even if they were present, intraspinal mechanoreceptors could not contribute information about leg movement. Moreover, in the present study, responses could be elicited to sensory stimuli applied to the legs only in chicks with incomplete lumbosacral deafferentations. Therefore, we feel confident that our deafferentation procedure eliminated all sensory feedback from the legs.

After lumbosacral deafferentation, behaviors with characteristics typical of hatching or walking could still be elicited. The aspects of the hatching leg motor output pattern that identified it as hatching were the episodic nature of the temporal organization and the synchronous interlimb coordination pattern. In contrast, when walking was elicited in a deafferented chick, the temporal organization of the leg motor output was cyclically repetitive and the interlimb coordination pattern was one of alternation. Thus, these characteristics of walking and hatching are not the result of modulation by sensory input from the legs.

The maintenance of differences in the temporal organization of the leg motor output patterns after deafferentation does not rule out the use of the same intralimb pattern-generating circuitry in both behaviors. The differences could result from the activation of one set of pattern-generating circuitry via different inputs. For example, it has been shown that the switch between 2 motor programs in the crayfish swimmeret system is, in part, due to input from interneurons that are active in different patterns, one episodic and one sinusoidal (Heitler, 1985). In this system, experimentally induced changes in the activity of a single interneuron can induce a switch from one motor program to the other. While this model is attractive, our data in the chick do not, at present, allow us to eliminate an alternative possibility. That is, the same input could activate different sets (or subsets) of pattern-generating circuitry, one that responds with an episodic temporal pattern and the other with a cyclically repetitive pattern.

The differences in interlimb coordination patterns that remain after deafferentation are most likely due to differences in the coupling between the pattern-generating circuits for each leg, rather than to differences in the intralimb pattern-generating circuits themselves. It has been shown in a variety of vertebrates, including chicks, that each leg has a separate pattern-generating circuit for locomotion (Jacobson and Hollyday, 1982a; see Grillner, 1981, for a review). In cats, for example, the circuits for the 2 hindlimbs can be coupled to produce an alternating interlimb pattern, as in a trot, or in a synchronous pattern, as in a gallop. Unfortunately, little is known about the precise mechanisms involved in switching gaits (Grillner, 1981). In chicks, descending input from sensory receptors in the neck appears to play an important role in the switch from an alternating pattern of interlimb coordination to the synchronous pattern seen during hatching (Bekoff and Kauer, 1982; Bekoff and Sabichi, 1987).

The remaining differences between the leg motor output patterns of hatching and walking involve intralimb coordination. After deafferentation, 2 general categories of changes are seen. In one group are those features of intralimb coordination that change in the same direction for both behaviors. For example, mean flexor burst durations increase significantly in both behaviors, while mean extensor burst durations typically change relatively little. Furthermore, when flexor burst durations are calculated as a percentage of the cycle period, similar changes are seen. Thus, the increases in flexor burst durations are not simply proportional to increases in cycle period. These results suggest that, under normal conditions, sensory input from the legs acts to limit flexor burst durations in both behaviors.

The second and more common type of change is convergence. For example, during walking before deafferentation, FT_a begins its activity well after the initiation of GL (phase FT_a, GL = 0.22). After removal of leg sensory input, it begins its activity at the onset of GL. This is a convergence with the hatching pattern, where FT_a begins its activity just at the onset of GL. A similar convergence is seen in IF_a.

Several other distinctive features of the intralimb motor output patterns of hatching and walking also become more similar after deafferentation. For example, one continuous burst of SA is seen during deafferented hatching, instead of the 2 bursts that are normally seen. Thus, this aspect of the hatching motor output pattern converges with the walking pattern.

The duration-versus-period relationships of flexors and extensors also converge after deafferentation. Specifically, the differential control of extensors and flexors seen during normal walking is lost; the extensor and flexor burst durations become equally sensitive to increases in cycle period. This feature of the walking motor output pattern becomes more similar to hatching.

In agreement with this finding, convergence of the slopes of the burst duration-versus-period relationships for extensors and flexors was also seen in a study of L-DOPA-induced fictive locomotion in chronic and acute spinal cats (Baker et al., 1984). As in lumbosacral deafferented animals, phasic sensory input from the legs is eliminated in fictive locomotion because of the

absence of movement. Evidently the tonic sensory input that is still available during fictive locomotion did not compensate for the absence of phasic input in this study. However, in another study of fictive locomotion in acute spinal cats, 12 of 14 recording sessions produced results in which the slope of the extensor burst duration-versus-period relationship remained steeper than the flexor slope (Grillner and Zangger, 1979). Nevertheless, in this study the relationships were reversed in one cat. That is, the flexor slope became steeper. In yet another study, in which locomotion was elicited in lumbosacral deafferented cats by stimulation of the mesencephalic locomotor region, the extensor slope remained steeper than the flexor slope, as is typically seen during normal walking (Grillner and Zangger, 1984).

While these results initially appear to be contradictory, they do show that, under some conditions, locomotion with the normal duration-versus-period relationships can be produced in both lumbosacral deafferented or fictive preparations. Nevertheless, under other conditions, the duration-versus-period relationships of extensors and flexors can converge in these preparations. In this context, it is interesting to note that while the extensor burst duration varies more than the flexor burst duration during tortoise walking (Williams, 1981), both extensor and flexor burst durations vary to a moderate extent during turtle swimming (Lennard and Stein, 1977).

Thus, our data on chicks, as well as the results from cats and turtles, suggest that whether or not extensor and flexor burst durations are differentially controlled relative to cycle period depends on the specific conditions under which the pattern-generating circuitry is activated. If this is the case, then maintaining the relative constancy of flexor burst durations is not an essential characteristic of the central pattern-generating circuitry for locomotion. Instead, these data suggest that the central pattern-generating circuitry for walking produces a basic motor output pattern in which both flexor and extensor burst durations increase to a moderate extent with increasing period. However, this aspect of the motor output pattern can be altered by modulatory mechanisms, one of which appears to be sensory input from the legs: under normal conditions, either tonic or phasic sensory input from the legs tends to bias the output of the central pattern-generating circuitry so that short and relatively constant flexor burst durations are produced during walking.

The nature of the specific sensory signal involved in limiting flexor burst duration is not known, although a recent study of swimming in chicks suggests that sensory information related to weight-bearing may be important (Bekoff, 1986). A role for load information in regulating extensor burst duration during walking has previously been suggested in cats (Duysens and Pearson, 1980; Giuliani and Smith, 1985). Because normal duration-versus-period relationships can be seen during locomotion in both fictive (Grillner and Zangger, 1979) and deafferented (Grillner and Zangger, 1984) cat preparations and were seen in 38% of the deafferented chicks in the present study, factors other than sensory input from the legs must also be able to bias this aspect of the output of the pattern-generating circuitry.

Convergence is also seen in the reciprocal duration-versus-latency relationships of GL and TA. That is, the asymmetrical coupling of onset and termination of GL and TA bursts seen during normal walking becomes symmetrical after deafferentation. This is a convergence to the hatching pattern, in which the relationship is symmetrical both before and after deafferentation.

The convergence seen in many features of the intralimb motor output patterns after deafferentation supports the hypothesis that the leg motor output patterns of hatching and walking are produced by the same intralimb pattern-generating circuitry. Specifically, our results suggest that sensory input from the legs normally modulates the output of this multiuse circuitry in order to produce many of the distinctive features of the 2 different intralimb motor output patterns of hatching and walking.

Moreover, the intralimb motor output pattern of hatching appears to be more basic. For example, the hatching leg motor output pattern is more symmetrical than the walking pattern. Overall, extensors and flexors behave more similarly during hatching: Burst durations are more similar; they vary to a similar, moderate extent with period; and there is a similar high correlation between the time at which the extensors turn off and the flexors turn on, and vice versa. In addition, when convergence is seen after deafferentation, it is usually the walking pattern that becomes more similar to hatching rather than the reverse. This suggests that the hatching motor pattern is closer to the basic, unmodulated output of the multiuse pattern-generating circuitry. Given that the hatching chick does not need to respond to, or compensate for, environmental variables to the same extent that a walking chick does, this appears to be a reasonable conclusion.

The changes seen after deafferentation may also provide insight into the role that sensory input normally plays in modulating pattern generator output. In general, following deafferentation, there are fewer and less dramatic changes in the motor output of extensor muscles than in flexors. For example, burst durations and phase relationships of flexors tend to show larger changes. The large increases in flexor burst durations (e.g., TA and SA) after deafferentation are correlated with a shift in their phase relationships, so they are activated earlier in the cycle, nearer the midpoint. In addition, the burst durations of these 2 flexors become more tightly correlated with period than they were prior to deafferentation. While these changes are larger for walking than for hatching, a substantial increase in the flexor slope and correlation coefficient are seen in both behaviors after deafferentation. These results suggest that sensory input from the legs normally modulates the motor output of flexors more strongly than that of extensors.

After deafferentation, there is also an increase in the variability of burst durations and cycle period, as measured by increases in the coefficients of variation. This suggests that another role of the sensory input may be to stabilize the output of the central pattern-generating circuitry.

While there is considerable convergence in intralimb motor output patterns, there are some distinctive aspects of the patterns that do not change after deafferentation. These correspond to the immutable elements in cat hindlimb motor patterns discussed by Smith and her colleagues (1986). For example, FT continues to be activated in a double-bursting pattern during walking, while a single burst is typical of hatching. Furthermore, the FT_b burst does not converge either with the flexor or extensor pattern after deafferentation. Thus, these data support a multipartite model of central pattern generation of intralimb coordination, rather than a bipartite model in which all flexors are activated together and in alternation with all extensors (e.g., Grillner, 1981; Jacobson and Hollyday, 1982a, b; Stein, 1986).

These results also suggest that while sensory input from the legs may be involved in modulating the output of a neural circuit for intralimb coordination so that it produces 2 different motor

output patterns, sensory input from the legs is not responsible for all of the differences. One of the remaining possible sources of modulatory input—descending input from the brain—can be eliminated on the basis of the results of spinal transection experiments (Bekoff, 1984; A. Bekoff, J. A. Kauer, A. Fulstone, and T. Anderson, unpublished observations).

Another possible source of modulatory input is sensory input from the cervical region. It is clear that input resulting from bending the neck plays a role in turning on the episodic motor activity and synchronous interlimb coordination that are characteristic of hatching (Bekoff and Kauer, 1982; Bekoff and Sabichi, 1987). It seems reasonable to propose that cervical input may also be responsible for modulation resulting in the unique aspects of intralimb motor output seen during hatching after sensory input from the legs and descending input from the brain have been removed.

According to this view, hatching leg motor output is produced when sensory input from the bent neck activates the basic intralimb pattern-generating circuitry of each leg in an episodic pattern. This input also couples the circuits of the 2 legs to produce synchronous interlimb coordination, and modulates them slightly. Sensory input from the legs further modulates the output of the basic intralimb circuits. Walking motor output is produced when the same basic intralimb circuitry is activated in the absence of the sensory signal from the bent neck. This results in a cyclically repetitive pattern of activation and in interlimb alternation. Sensory input from the legs modulates the basic intralimb circuitry. Modulation by some other, as yet unexplored, source of sensory input may be responsible for some of the characteristic aspects of the intralimb motor output pattern of walking that are not lost after limb deafferentation.

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