Developmental Changes in Leg Coordination of the Chick at Embryonic Days 9, 11, and 13: Uncoupling of Ankle Movements

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Sharp, Andrew A., Edna Ma, and Anne Bekoff. Developmental changes in leg coordination of the chick at embryonic days 9, 11, and 13: uncoupling of ankle movements. J. Neurophysiol. 82: 2406–2414, 1999. To understand changes in motor behavior during development, kinematic measurements were made of the right leg during embryonic motility in chicks on embryonic (E) days 9, 11, and 13. This is an interesting developmental period during which the embryo first becomes large enough to be physically constrained by the shell. Additionally, sensory systems are incorporated at that time into the spinal motor circuitry. Previous electromyographic (EMG) recordings have shown that the basic pattern of muscle activity seen at E9, composed of half-center-type alternation of extensor and flexor activation, breaks down by E13. This breakdown in organization could be because of disruption of motor patterns by the immature sensory system and/or new spatial constraints on the embryo. The current article describes several changes in leg movement patterns during this period. Episodes of motility increase in duration and the intervals of time between episodes of motility decrease in length. The range of motion of the leg increases, but the overall posture of the leg becomes more flexed. It was found that in-phase coordination of movement among the hip, knee, and ankle decreased between E9 and E13 in agreement with the previous EMG recordings. However, it was also found that the decrease of in-phase coordination among the three joints was accompanied by an increase in the time any two joints were moving in the same manner. Furthermore, examination of in-phase coordination within pairs of joints showed that all three pairs were well coordinated at E9, but that at E13 the in-phase coordination of the ankle with the other two joints decreased, whereas the knee and hip coordination was maintained. This suggests that the hip-knee synergy was closely coupled and that coupling of the ankle with other joints was more labile. The authors conclude that embryos respond to the reduction of free space in the egg during this period not by decreasing the amplitude or coordination of leg movements in general, but instead by differentially controlling the movements of the ankle from those of the hip and knee. Additionally, the changes in movement patterns do not represent a decrease in organization, but rather an alteration of motor coordination possibly as the result of information from the newly acquired sensory systems. These data also support theories that limb central pattern generators (CPGs) are composed of unit CPGs for each joint that can be modulated individually and that this organization is already established early in embryogenesis.

INTRODUCTION

Studies on embryonic motility in the chick have yielded many insights into the development of coordinated movement in vertebrates. For example, embryonic motility is neurogenic (Ripley and Provine 1972) and centrally generated, not strictly reflexogenic (Hamburger et al. 1966; Oppenheim 1966). The early behavioral descriptions of embryonic motility as starts and wriggles (Hamburger and Oppenheim 1967) lead to the belief that there is little or no underlying coordination of the nervous system generating this behavior until hatching is initiated. However, kinematic and electromyographic (EMG) analyses have shown that this is not entirely true. Kinematic recordings of E9 and E10 embryos have shown coordination of extension and flexion among the joints of the right leg (Bradley 1997; Chambers et al. 1995; Watson and Bekoff 1990). Cyclic repetition of extension and flexion is common. It has also been demonstrated that there is coordination between the joints of the wing and leg (Bradley 1997; Chambers et al. 1995). Watson and Bekoff (1990) have suggested that it is the variability in the number of joints active during a movement and the variability in frequency and amplitude of movements that give the impression that embryonic motility is uncoordinated.

EMG recordings from the muscles of the right leg during embryonic motility show that there is an underlying pattern of coordination during motility. This supports the findings of the kinematic studies. Recordings at E7 (Bekoff 1976) and E9 (Bekoff 1976; Bradley and Bekoff 1990) show that early embryonic motility is characterized by a half-center-type alternation of extensor and flexor muscle synergies. The basic pattern, which is established by E9, appears to form the basis for mature motor patterns, such as hatching and walking (Bekoff 1992). Interestingly, the embryonic motor patterns do not show a smooth, linear trajectory between E9 and hatching on E21. Instead, the motor patterns become much less coordinated by E13 but then return to a higher level of coordination by E17 (Bekoff 1976).

There are a number of events occurring between E9 and E13 that may contribute to the decrease in motor output coordination. By E11 the embryo has become large enough that it starts to fold its legs in response to the limited space within the egg. There are also changes in the sensory systems occurring at this time. At the end of E7, monosynaptic connections between proprioceptive neurons and motor neurons are first formed (Davis et al. 1989; Lee et al. 1988). Massive branching of the proprioceptive inputs occurs between then and E13. Additionally, reflex responses to flipping the end of the limb can be seen as early as E7.5 and to stroking of the limb as early as E8.5 (Oppenheim 1972). Hamburger and colleagues (1981) also determined that normal sensory neuron cell death occurs between E4 and E13. Both spinal and supraspinal neurotransmitter systems are also changing dramatically during this period.
Experimental conditions

Eggs were opened by making a lateral window in the shell. They were then placed in a temperature-controlled (37°C), humidified recording chamber. The chorioallantoic and amniotic membranes were opened to expose at least the posterior half of the embryo so that the right leg was visible. Care was taken to cut as little of the extraembryonic vasculature as possible. The experiment was terminated if there was excessive bleeding, the heart rate dropped below 120 beats/min, or the embryo did not move at least once within a 5-min period (Bradley and Bekoff 1990).

Typically, embryos of this age lie on the left side, with the right leg easily visible, but rotate out of the x-y plane during motility (Chambers et al. 1995). To limit this type of movement, which confounds kinematic measurements, each embryo was glued to two rigid supports with Vetbond Tissue Adhesive (3M, St. Paul, MN). The supports were made on E9, E11, or E13. At the completion of each experiment, the embryos were killed with CO2 gas (American Veterinary Medical Association 1997).

Statistical analysis

Ten consecutive minutes of activity were used from each of six animals at each age. From each of these records five episodes of leg motility were selected for detailed analysis. We compared sample means among measures using ANOVA. If a significant overall result was obtained, we then used the Tukey-Kramer post hoc test for multiple comparisons. Percentages were normalized using the $\sin^{-1} \sqrt{P}$ transformation before ANOVA. $P < 0.05$ was required for statistical significance.

RESULTS

Embryonic environment

We studied embryonic motility at E9, E11, and E13. Figure 1 shows tracings from video frames at these stages illustrating the growth of the embryo and the relative sizes of the embryos in comparison with the limitations of the egg. At E9 the embryo had sufficient room within the egg to extend its legs fully without encountering the yolk sac. At E11 the embryo was large enough that it could not fully extend its legs without pressing into the yolk sac. The limitations of space at E13 were placing small spots of fingernail polish (Wet ‘n’ Wild, Nyack, NY) onto the back (above the spinal cord, midway between the hip and shoulder), hip, knee, ankle, and the tarsal-metatarsal junction (Watson and Bekoff 1990). Fingernail polish was applied with a fine syringe that had been equipped with a short length of flexible tubing.

Motility was videotaped with a S-VHS color video camera (Panasonic WV-CL700), mounted on a dissecting microscope (model M3Z, Wild Heerbrugg Instruments, Heerbrugg, Switzerland). Each embryo was recorded for 10–20 min at 60 frames/s. The videotapes were subsequently viewed frame by frame to generate a log of start and stop times for motility of the right leg. We defined a movement episode as any movement in the right leg that was separated from other right leg movements by at least 10 s of quiescence in that leg. This is similar to previous studies (e.g., Bradley 1997; Oppenheim 1975). Approximately 10 min of motility was analyzed for each animal (6 animals at each age) to determine the mean movement episode duration and interepisode interval. The 10-min period was taken from the beginning of a movement episode to the end of either the movement episode or the interepisode interval that was in progress 10 min later. This resulted in a sample of somewhat more than 10 min of actual time for each animal.

Five motility episodes from each embryo were digitized for joint angle measurements using a Peak Motus system (Peak Performance Technologies, Englewood, CO). Because no significant differences were seen between records sampled at 10 frames/s or at the full 60 frames/s, data were analyzed from videotapes sampled at 10 frames/s. Joint angle versus time was plotted for the hip, knee, and ankle for each digitized record. In addition, maximum, minimum, and resting angles were calculated for each joint from each digitized record.

M E T H O D S

Animals

Fertile White Leghorn eggs were obtained from SPAFAS (Preston, CT). They were incubated under standard conditions in a rotating, forced-air incubator (Humidade Incubator, New Madison, OH). Eggs were placed on their sides in a stationary incubator (Leahy Manufacturing, Higginsville, MO) for 12–24 h before experimentation so that embryos would rotate to the top for better accessibility. E0 was defined as the day the eggs were incubated. Recordings were made on E9, E11, or E13. At the completion of each experiment, the embryos were killed with CO2 gas (American Veterinary Medical Association 1997).

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It was not possible to view all the joints of the right leg at all times, because the yolk sac often covered part of the embryo. In these cases, fine wires were hung over the side of the shell to retract any membranes or the yolk sac overlying the leg. Embryos were not allowed to contact these wires during the course of motility, and experiments were only recorded if it was possible to provide a natural orientation of the embryo with all joint markers clearly visible. Preparation of the embryo was such that it neither increased nor decreased the normal spatial constraints placed on the embryo.

The hip, knee, and ankle angles of the right leg were defined by placing small spots of fingernail polish (Wet ‘n’ Wild, Nyack, NY) onto the back (above the spinal cord, midway between the hip and shoulder), hip, knee, ankle, and the tarsal-metatarsal junction (Watson and Bekoff 1990). Fingernail polish was applied with a fine syringe that had been equipped with a short length of flexible tubing.

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FIG. 1. Embryos at E9, E11, and E13. All tracings are at the same scale.
still greater. The embryo was obviously more flexed. Even if
the yolk sac were not present, it could no longer extend its legs
fully within the shell. Furthermore, E13 embryos did not ex-
tend their legs as far as is possible. The legs were never seen
to push directly against the shell.

Episodic motility

As in previous studies (Chambers et al. 1995; Hamburger
and Balaban 1963; Hamburger et al. 1965; Watson and Bekoff
1990), we found that embryonic motility is typically episodic
with periods of movement separated by periods of quiescence.
In the current study we examined episodes of right leg move-
ments. We found that the frequency distributions of episode
durations changed during development (Fig. 2). At E9 the
distribution was bimodal with one group of values clustered at
$\leq 5$ s and the second group distributed around the mean value
of 29 s. For E11, the distribution changed so that the episodes
were more evenly distributed between 0 and 90 s. There were
also episodes that lasted more than the 65 s maximum duration
seen at E9. The distribution of episode durations at E13 was
quite different. The values were more widely distributed, with
episodes up to 12 min (values $> 150$ s not shown). The mean
values for episode duration were $29 \pm 10$ (SD) s, $50 \pm 24$ s,
and $142 \pm 122$ s for E9, E11, and E13, respectively. In general,
the mean episode duration increased from E9 to E13. There
was a significant change in the mean for E9 versus E13 and
E11 versus E13, but not E9 versus E11.

Joint angle measurements

Joint angles were determined for the ankle, knee, and hip
joints as described in METHODS. Five episodes of activity were
selected for each of six animals at E9, E11, and E13. For E9
and E11 animals episodes with durations similar to the group
mean were selected. Because the episode durations for the E13
animals were so widely dispersed and few were near the mean,
it was necessary to select episodes differently than for the E9 and E11 embryos. We chose to use five segments of motility, each of which ranged from 50 to 70 s in length, from each animal. Some were complete motility episodes, and for others 60 s of activity were selected from longer motility episodes. This allowed us to normalize the amount of information provided from each embryo while still providing a representative sampling of motility patterns.

One representative joint angle plot for each of the three embryonic ages is shown in Fig. 3. The range of motion of the ankle, knee, and hip changed during development. We measured the maximum, minimum, and resting angles for each motility episode. Resting angles were measured at the start of each motility episode. The mean values are plotted in Fig. 4, and the statistically significant differences are shown in Table 1. The most marked changes were seen at the ankle. The total ankle joint excursion (difference between the mean maximum and minimum joint angles) became significantly larger between E9 and E11. This change was accompanied by a significant decrease in the maximum, minimum, and resting angles. Ankle joint excursion did not change significantly between E11 and E13. However, the minimum angle continued to decrease.

![Fig. 3](http://jn.physiology.org/)

**FIG. 3.** Representative joint angle vs. time plots for individual motility episodes for A E9, B E11, and C E13 embryos. The joint angles for the hip, knee, and ankle are shown.
were many events during an episode of activity that were either neously moving in the same manner (either moving synchro-
during which all joints, two joints, or no joints were simulta-
method to categorize the percentage of each motility episode
the movements of the leg joints. To this end we devised a
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down of in-phase coordination seen at E11 and E13 is the result
older. We wanted to determine whether the apparent break-
became increasingly difficult to recognize as the embryos got
same direction) of cyclical extension and flexion events
angle plots in Fig. 3 illustrate that in-phase, interjoint coordi-
mately one-half of the cycle (Fig. 3A). However, the joint
angle decreased, which resulted in the joint excursion returning to the same magnitude as at E9 but over a more flexed range.
The changes seen at the hip joint were very different from those seen at the knee and the ankle. The excursion magnitude of the hip did not change in this developmental time frame, but the range became significantly more flexed at E11. Interestingly, the range returned to the E9 level at E13.

Interjoint coordination

Recordings of E9 embryos show that there were times when there were cyclical alternations of extension and flexion at the hip, knee, and ankle with each synergy occupying approximately one-half of the cycle (Fig. 3A). However, the joint angle plots in Fig. 3 illustrate that in-phase, interjoint coordination (defined as simultaneous movements of the leg joints in the same direction) of cyclical extension and flexion events became increasingly difficult to recognize as the embryos got older. We wanted to determine whether the apparent breakdown of in-phase coordination seen at E11 and E13 is the result of a general, random disruption of in-phase coordination or whether there is still some underlying pattern of organization to the movements of the leg joints. To this end we devised a method to categorize the percentage of each motility episode during which all joints, two joints, or no joints were simultaneously moving in the same manner (either moving synchronously in the same direction or not moving).
The first step in this process was to define when a change in joint angle is considered to be a significant movement. There were many events during an episode of activity that were either of very low amplitude or of very rapid time course. These events were presumably the result of a variety of nonrelevant processes such as minor digitizing error, incidental movements resulting from the heart beating, or random, low-level discharge from motor neurons. To determine criteria that could be uniformly applied to recordings, A. A. S. and A. B. independently marked all joint angle changes they considered to be movements in three recordings from each of three E9 animals. This resulted in a >95% agreement. The measurements made by A. A. S. were then used to set the criteria. Three measurements for each significant and nonsignificant movement were made: the change in angle \( \Delta \), the time it took to reach maximum displacement \( T_M \), and the time it took to return to the original joint angle \( T_R \). If \( T_R > 2 \) s, it was recorded as 2 s.

\( T_M \) and \( T_R \) were then plotted versus angle \( A \) for each joint. Figure 5, A and B show the plots for the ankle. The movement and nonmovement events fell into two distinct groups that could be separated by a simple time and amplitude rule that was slightly different for each joint. If the amplitude of an event was greater than a given value (10° for the ankle) and \( T_M > 0.25 \) s for the ankle) it was considered a movement. However, if \( A > 10.0° \); knee: \( T_M = 0.25 \) s, \( T_R = 0.70 \) s, \( A = 8.0° \); hip: \( T_M = 0.25 \) s, \( T_R = 0.70 \) s, \( A = 7.5° \).
The records from the three joints were then compared to determine what percentage of each motility episode was composed of all three joints, two joints, or no joints performing the same motion. The results are summarized in Fig. 6A. At E9, ~90% of each episode was represented by in-phase coordination of either two joints or all three joints. Each of these groups occupied ~45% of the episode. Only ~10% of each episode lacked this type of coordination among the joints. At E11 there

![Graph showing joint angles for E9, E11, and E13 embryos.](image)

**TABLE 1. Summary of pairwise comparisons between age groups for joint angle properties**

<table>
<thead>
<tr>
<th>Joint/Property</th>
<th>E9 vs. E11</th>
<th>E9 vs. E13</th>
<th>E11 vs. E13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum angle</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Minimum angle</td>
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<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Rest angle</td>
<td>S</td>
<td>S</td>
<td>NS</td>
</tr>
<tr>
<td>Joint excursion</td>
<td>S</td>
<td>S</td>
<td>NS</td>
</tr>
<tr>
<td>Knee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum angle</td>
<td>S</td>
<td>S</td>
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<td>Minimum angle</td>
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<td>Rest angle</td>
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<td>Joint excursion</td>
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<tr>
<td>Hip</td>
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<tr>
<td>Maximum angle</td>
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<td>Minimum angle</td>
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<td>Rest angle</td>
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<tr>
<td>Joint excursion</td>
<td>NS</td>
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<td>NS</td>
</tr>
</tbody>
</table>

Statistical comparisons were made pairwise using the Tukey-Kramer test. S, significantly different, \( P < 0.05 \); NS, not significant, \( P > 0.05 \).
was a significant decrease in the in-phase coordination of all three joints and a corresponding significant increase in two-joint, in-phase coordination. There was no change in the percentage of each episode when all three joints were moving differently from one another. These changes persisted at E13.

We were mainly interested in knowing whether the joint movements showed in-phase coordination during muscle activation. The all-joints-in-phase category included times when all the joints were showing sustained positions. If all the joints were maintaining a resting value, then the muscles were most likely not activated. We therefore recalculated the values after we had removed the time when all the joints were at rest. The results are shown in Fig. 6B. At E9, all three joints were in phase ~30% of the time. Two joints were in phase ~60% of the time. It was apparent that there were no significant changes of in-phase coordination until E13. At E13 the percentage of time in which all three joints were in phase decreased significantly, whereas there was a corresponding significant increase in the two-joints-in-phase category.

To determine the level of in-phase coordination within pairs of joints, we plotted the total percentage of time any pair of joints was performing the same motion (Fig. 7). For example, the total time when the ankle and knee were in phase was the time when only the ankle and knee were in phase plus the time when all three joints were in phase. This calculation does not include times when all three joints were at rest. There was no significant change in the pairwise coupling among the joints from E9 to E11. There was also no change in the total knee and
hip coupling at E13. However, there was a significant decrease in coupling between the ankle and knee as well as the ankle and hip at E13.

**DISCUSSION**

In this study we examined the changes in motility exhibited by embryonic chicks at E9, E11, and E13. This is a period when the sensory-motor system is undergoing many reconfigurations, and the embryo has also grown sufficiently to start to be spatially restricted by the shell. We were particularly interested in knowing how the apparent decrease in organization of the motor patterns seen in EMG recordings (Bekoff 1976) would be reflected in the actual behavior of the animal.

**Episodic motility**

In accordance with previous studies that have focused on either movements of all parts of the body (e.g., Hamburger and Balaban 1963; Hamburger et al. 1965), the legs and wings (Bradley 1997; Chambers et al. 1995) or just legs (Watson and Bekoff 1990), we found that motility of the right leg was episodic with periods of rest between periods of motility. As the embryo developed from E9 to E13 it moved its leg for longer times and rested for shorter periods. In fact, the percentage of time the embryo was active increased from 36 to 84%. This occurred in a two-stage process. First, by E11 the embryos had decreased the amount of time they were resting between episodes of motility. Second, by E13 they had greatly increased the duration of each motility event. The fact that this change occurred as a two-stage process is significant, because it suggests that the mechanisms that control initiation of motility and maintenance of motility may be regulated separately or by interacting processes.

The increase in activity seen between E9 and E13 has previously been reported (Hamburger et al. 1965). It is interesting that they saw a very similar change in total activity (39–80%), although they were looking at movements of all body parts, whereas we examined only right leg movements. However, Hamburger and colleagues (1966) reported lower mean episode durations and lower mean interepisode intervals, except at E13 when they were approximately the same. These differences were most likely the result of different methods of defining movement.

The increase in activity of the embryos between E9 and E13 may be the result of increased excitability of the motor central pattern generator (CPG). This could be because of intrinsic changes in the spinal circuitry or to maturation of modulatory inputs from sensory and descending systems. It seems likely that supraspinal input is a contributing factor. For example, there is an increase in serotonergic fibers in the spinal cord at this time (Sako et al. 1986). Oppenheim (1975) found that embryos with cervical gaps first showed a reduction in activity at E10. Additionally, Bradley and Bekoff (1992) found a decrease in burst duration and cycle period measured from EMG recordings of the right leg muscles in embryos with thoracic spinal transections.

Immunohistochemical evidence shows that there are also significant changes in the spinal transmitter systems occurring at this time. Spinal neurons expressing glycine (Berki et al. 1995), serotonin (Sako et al. 1986), substance P (Du et al. 1987), enkephalin (Du and Dubois 1988), and calcitonin gene-related peptide (Carr and Wenner 1998) are increasing in number. The number of GABAergic neurons is increasing in the dorsal horn and decreasing in the ventral horn (Berki et al. 1995). Such major reconfiguration of the spinal transmitter systems is undoubtedly playing some role in the observed behavioral changes at this time.

Some studies have suggested that although afferent connec-
tions have been made by E7.5 (Davis et al. 1989; Lee et al. 1988), sensory input may not play a role in ongoing embryonic movements. For example, Hamburger and colleagues (1966) reported that deafferentation achieved by removing the dorsal half of the lumbar-sacral spinal cord did not alter the amount or the appearance of the activity. However, kinematic techniques, which allow detailed quantitative analysis of movements, were not available at the time of that study. It remains possible that kinematic analysis will reveal changes not detected by their methods. Oppenheim (1972) has shown that embryos quickly cease responding to tactile or proprioceptive stimuli, suggesting that they may habituate rapidly. Nevertheless, this does not answer the question of whether they use sensory input during self-initiated movements. Suggestive evidence for sensory modulation has come from two studies that have shown that a reduction of buoyancy alters embryonic motility (Bradley 1997; Chambers et al. 1995). However, an evaluation of precisely how sensory modulation is involved in the control of embryonic activity necessitates more specific sensory ablations and more detailed analyses of the effects.

Joint excursions

The position and range of motion displayed by the leg changed markedly between E9 and E13. Our measurements of joint position and excursion at E9 were very similar to those reported by Watson and Bekoff (1990) who showed that at E9 the leg of the embryo was markedly extended. At E11, we found that all three joints were more flexed than at E9, and the amplitude of excursion of the knee and ankle had increased. By E13 the position of the hip had returned to the E9 position, but the ankle and knee remained flexed. In general, the leg became more flexed between E9 and E13, and the range of motion of the ankle became significantly larger. The increased range of motion was probably due to either an increase in motor neuron output or an increase in muscle strength. However, the increased general flexion of the embryo was probably a necessary accommodation for the reduction in free space surrounding the growing embryo. If the feet pressed against the shell, the embryo might damage the extraembryonic membranes. This suggests that the embryos were able to use sensory information about their environment to avoid destroying the integrity of their membranes.

Interjoint coordination

Our time series plots of joint angles reveal the same type of activity at E9 as has previously been reported (Bradley 1997; Chambers et al. 1995; Watson and Bekoff 1990). There were periods when cyclic alternations of extension and flexion events exhibited in-phase coordination (moving in the same direction) among the joints. Many movements involved some combination of two joints moving at the same time in the same direction. The amplitude of the motions also showed great variability. At E11 and E13 it became increasingly difficult to discern obvious cyclical alternations of coordinated extension and flexion events. This is consistent with the decrease in organization of motor output revealed by EMG recordings (Bekoff 1976).

The basic motor pattern seen at E9 suggests that there is a tight relationship between the extensor and flexor synergies of the leg (Bradley and Bekoff 1990). We examined whether the interjoint coordination patterns seen at E9 were maintained at E11 and E13, despite the apparent disruption of the EMG pattern. We analyzed the coordination for entire motility episodes and for the portion of motility episodes when all three joints were active. Both analyses yielded the same general finding. Between E9 and E13 the percentage of time when all three joints were coordinated decreased, whereas the time when only two joints were coordinated increased. The decrease in coordination among all three joints is most likely one behavioral result of the loss of organization seen in E13 EMG recordings (Bekoff 1976). The apparent lack of coordination reported in earlier studies (e.g., Hamburger and Oppenheim 1967) may be in part because most movements showed coordination between only two joints.

When the coordination between pairs of joints was examined we observed something very interesting. Although the coordination between the ankle and other joints was reduced between E9 and E13, the coupling between the knee and hip did not change. Although the findings of an earlier EMG study (Bekoff 1976) suggested a general disorganization of the motor output, that study used only recordings from muscles controlling the ankle and knee. Therefore, our kinematic data suggest that the coupling of the knee and hip movements was tightly regulated by the nervous system, but that the ankle synergies were more labile. This is particularly interesting because the joints closest to the base of support are generally the site of greatest accommodation after perturbation in adults (Farley et al. 1998; Horak and Nashner 1986; Tang et al. 1998).

In summary, we suggest the following explanation for the changes in motility seen between E9 and E13. As the embryo matures between E9 and E13, it becomes more spatially constrained by the shell. By E13 it is no longer possible for the embryo to fully extend its leg. Despite this, the excursion of the ankle is increased. This is accompanied by a more flexed posture of the knee and ankle. If the embryo were not able to use sensory information, we would not necessarily expect to see these specific changes in positioning of the limbs. Instead we would expect to see a variety of different motility patterns that reflect different contact points and that would show alterations of coordination in all possible joint combinations. Also, if the immature sensory systems were disrupting locomotor patterns we would expect a general disorganization of motility. Therefore, it seems likely that the sensory systems are sufficiently integrated into the motor CPGs to allow sensory information to be used to alter motility in accordance with the spatial constraints experienced by the embryo. Specifically, the movement of the ankle is differentially controlled from that of the knee and hip. In addition, the embryo’s descending modulatory pathways and sensory systems have also become more mature and integrated into the concomitantly developing spinal motor circuitry. As a result of these changes the embryo becomes more active and demonstrates a wider range of motions.

This result is in keeping with Coghill’s (1929) theory of motor development, which suggests that specific adaptive movements individuate from more global “total” movements. At E9, adjacent joints showed a high degree of coordination, but by E13 the coordination of the ankle with any other joint had markedly decreased. Furthermore, these data are consistent with the idea that there may be separate CPGs for each joint.
and that they may be differentially controlled depending on circumstances (Grillner 1981). Our data suggest that this type of CPG organization may already be in place at E9 and that sensory information from a constrained environment in the shell causes the embryo to selectively decrease the coupling of the ankle CPG with the other joint CPGs during embryonic motility.

Future studies using synchronous kinematic and EMG recordings are needed to determine the precise relationship between leg movements and muscle activity patterns. The current study suggests that focusing on how the coordination of ankle muscle activity with knee and hip muscle activity changes during development and in response to sensory perturbations would be useful.

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