Transformations in Embryonic Motility in Chick: Kinematic Correlates of Type I and II Motility at E9 and E12

NINA S. BRADLEY
Department of Biokinesiology and Physical Therapy, University of Southern California, Los Angeles, California 90033

Bradley, Nina S. Transformations in embryonic motility in chick: kinematic correlates of type I and II motility at E9 and E12. J. Neurophysiol. 81: 1486–1494, 1999. Soon after hatching, chicks exhibit an array of adaptive, coordinated behaviors. Chick embryos also acquire nearly 18 days of movement experience, referred to as embryonic motility, before hatching. The chick expresses three forms of motility, types I, II, and III, and each emerges at a different stage of embryonic development. Although much is known about the mechanisms associated with motility at early embryonic stages and at the onset of hatching, the transformations in behavior and underlying mechanisms are not fully understood. Thus the purpose of this study was to determine how motility is modified during the first expected transformation, from type I to type II. It was hypothesized that kinematic features for motility at embryonic day 12 (E12) would differ significantly from features at E9 because type II motility emerges during E11. Embryos were video taped for extended intervals in ovo at E9 or E12 and entire sequences of motility were computer digitized for kinematic analyses. Results reported here indicate that several of the kinematic features characteristic of motility at E9 are also reliable features at E12. On the basis of these findings, a kinematic definition of type I motility is posed for use in subsequent behavioral studies. Several parameters distinguished motility at E12 from E9. The most notable difference between ages was the less regular timing of repetitive limb movements at E12, a finding consistent with recent reports suggesting early motility is an emergent product of a transient neural network rather than a specialized pattern generator. As predicted from established definitions for type II motility, startle-like movements were common at E12; however, they also were present in many kinematic plots at E9, suggesting the discreet age-dependent boundaries in the established definition for type II motility may require modification. Some age-related differences, such as increased intralimb coordination and excursion velocity, may be prerequisites for adaptive behavior after hatching.

INTRODUCTION

Within hours after hatching, chicks initiate several adaptive behaviors, including walking (Jacobson and Hollyday 1982), running (Muir et al. 1996), airstepping (Johnston and Bekoff 1996), scratching (Smith and Bekoff 1990), and foot shaking (Woolley et al. 1990) that are likely produced by pattern generators (Bekoff 1992). The chick also has several days of movement experience in ovo before hatching, and until recently it was hypothesized these early movements also were produced by a developing pattern generator for adaptive locomotion (O’Donovan et al. 1992). More recent discussion has suggested that spontaneous motility is the product of (spinal neuron) population behavior emerging from transient network properties unique to early embryogenesis rather than a specialized pattern generator (O’Donovan and Chub 1997). Whether a transient network or an immature network undergoing refinement for eventual adult behaviors produces motility, the possibility remains that early movement experiences in ovo contribute importantly to the emergence of adaptive behaviors after hatching (Hall and Oppenheim 1987). It is well established that motility is important during embryogenesis for differentiation and maturation of the musculoskeleton (Drachman and Sokoloff 1966; Hall and Herring 1990; McLennan 1983; Toutant et al. 1979) and also may be important for establishment of spinal circuitry (Fields and Nelson 1992; Kalb and Hockfield 1992). For a more historical perspective on the function and central regulation of embryonic motility, the reader may wish to consult several additional studies (Hamburger et al. 1966; Haverkamp and Oppenheim 1986; Oppenheim 1973, 1975, 1984; Oppenheim et al. 1978; Shimazu et al. 1990).

In the definitive study of embryonic movements in the chick, Hamburger and Oppenheim (1967), using direct observational methods, identified three forms of behavior in ovo, types I, II, and III motility. They defined type I motility as random, jerky, small amplitude movement, and on the basis of direct observational methods, they concluded that these movements did not appear to be coordinated across body regions, such as the legs, wings, and head. Type II motility was defined as sudden, rapid wriggles and startles of the whole body; observations indicated that instances of type II motility were followed by type I motility. Type III motility was defined as prehatching and hatching movements (tucking and piping of the egg shell) and was viewed as the first coordinated, goal-oriented behavior produced by the embryo. Their findings indicated that type I motility emerged with the onset of movement at embryonic day (E) 3.5 and continued through the end of incubation, whereas type II motility emerged E11 and type III emerged E17.

The boundaries drawn by the above definitions suggest that each motility type is a unique behavior with a distinct developmental time course. During the period between E9 and E12, when the first behavioral transformation is expected, a number of important events occur. By E9–E10, 1a afferents monosynaptically contact both homonymous motor neurons (Lee et al. 1988) and motor neurons to synergist muscles (Lee and O’Donovan 1991). Collateral 1a axons achieve their greatest longitudinal extent (20 segments) and begin to retract to a final projection range of 14 segments (Eide and Glover 1995). Reticulospinal and vestibulospinal projections, having first reached the lumbosacral cord between E5 (reticulospinal) and

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E8 (rubrospinal), approximate adult-like distribution patterns by E10 and only the cerebellospinal projection appears to be absent (Okado and Oppenheim 1985). Serotonergic projections from the raphe nuclei reach the lumbar cord by E8 (Okado et al. 1992) and by E12, application of serotonin induces depolarizations in ~50% of lumbosacral neurons (Muramoto et al. 1996). Regional differentiation of spinal network activity associated with motility can be detected by E9, for in the isolated cord, rostral segments exert an excitatory influence on caudal segments where as caudal segments exert an inhibitory influence on rostral segments (Ho and O’Donovan 1993). Given the array of changes occurring within this time period, it is likely transformations in the control of motility are also occurring. For example, the different reciprocal influence of rostral and caudal spinal networks suggests region-specific changes in control of limb movements may emerge between E9 and E12 and may be detectable during motility.

In an earlier study, kinematic features that were common across embryos during motility at E9 were identified, and findings suggested, contrary to direct observational studies, that type I motility is an orderly, coordinated behavior (Chambers et al. 1995). In this study, it was hypothesized that kinematic features for motility at E12 would differ significantly from features at E9. Thus the purpose of this study was to identify the kinematic parameters of motility at E12 and determine how motility is modified during a period when transformations are expected. Results suggest that motility at E12 retains many of the characteristics of motility at E9 and that it is an orderly behavior with reliable features. Based on the consistencies identified between ages, a kinematic definition of type I motility is posed for reference in subsequent behavioral studies. Also the findings suggest that the discreet age-dependent boundaries of the established definitions for motility in the chick may require modification. The kinematic measures of embryonic motility presented here provide a foundation for further examining how self-initiated behavior changes over the embryonic period of development and also provide behavioral references for studying motility-related activity in reduced preparations between E9 and E12. An abstract of this work recently was published (Bradley 1998).

**Methods**

Leghorn chicken eggs were incubated for experiments at either E9 or E12, and staging criteria were used to verify age (Hamburger and Hamilton 1951). Standardized procedures, previously established (Chambers et al. 1995), were employed to insure optimal behavioral recordings. These procedures restricted the amount of preparation time and total exposure time per embryo. The procedures also specified, based on parameters indicative of deteriorating viability (i.e., pulse rate), when experiments were to be terminated. All procedures were approved by the university institutional review board monitoring use of animals.

**Preparation for kinematic recording**

A window was made in the eggshell to view the full sagittal extent of the embryo. Detailed accounts of the kinematic data collection procedures were described previously (Chambers et al. 1995; Orosz et al. 1994). Briefly, dots of nail enamel were placed on the skin or fascia of the right wing and leg, approximating joint location for the shoulder (humeroscapulocoracoid), elbow (humeroradioulna), wrist (dorsal carpal), hip (coxofemora), knee (femorotibiofibula), ankle (tibiometatar-arsa), and tarsometatarsus (Fig. 1A). An additional dot was placed along the thoracolumbar spine, a reference marker was placed on the shell dorsal to the thoracolumbar marker, and a 5-mm reference stick was floated on the surface of the amniotic fluid. Video recording was continuous for a maximum of 1 h, capturing all activity and pauses in activity. Camera shutter speed was 1/2,000 s to minimize blur of joint markers during movement.

**Kinematic data analyses**

The beginning and end of each motility sequence and the subsequent pause were determined during video playback. Adapting methods for direct observation established by Hamburger and colleagues (Hamburger et al. 1965), a motility sequence was defined as continuous movement of the right wing and/or leg and included all pauses in movement lasting 1–10 s (Fig. 1B, asterisks). A motility pause was defined as absence of detectable excursions in the right limbs exceeding 10 s. Movements lasting <10 s were included in the motility pause, as they were often barely discernible and therefore less reliably detected during video review. The combination of a motility sequence and subsequent motility pause formed one motility episode. The left limbs were ignored because they were typically out of view.

A total of five motility sequences per embryo were selected for digitizing. Selection of sequences was based on requirements for computer-automated digitizing and correcting out-of-plane movement...
RESULTS

Eight E12 embryos met viability and kinematic criteria for analyses. Eight E9 embryos also met these criteria; 7 of the E9 embryos also were used as a control group in another study conducted concurrently, examining the effects of buoyancy on motility at E9 (Bradley 1997). Total recording time for E12 embryos ranged from 20 to 50 min, averaging 34 ± 10 (SD) min, and the sample was not different from that for E9 embryos (33 to 53 min, P < 0.09).

Sampling of behavior and episode characteristics

The entire video recording for each embryo was reviewed to determine onset and duration of all motility sequences, motility pauses, and episode duration (sequence and subsequent pause). Overall, these analyses indicated that sequences were slightly longer and pauses substantially shorter for E12 embryos compared with E9 embryos (Fig. 2). Sequence duration averaged 44 ± 14 s for E12 embryos and 33 ± 5 s for E9 embryos (P < 0.03). Pauses between sequences averaged 52 ± 22 s for E12 embryos and 163 ± 51 s for E9 embryos (P < 0.0002). As pauses were substantially shorter for E12 embryos, total episode duration was also less, averaging 97 ± 14 s compared with 195 ± 50 s for E9 embryos (P < 0.0004). Normalized with respect to the concurrent episode duration, motility sequences for E12 embryos ranged from 31 to 74% of the episode (48 ± 13%) compared with a range of 11 to 30% (18 ± 6%) for E9 embryos (P < 0.0005).

In previous studies of E9 embryos, movements lasting <10 s were excluded from data analyses. Observations during E12 experiments, however, suggested that brief, abrupt movement was a common feature during both motility sequences (Fig. 3) and motility pauses. As it was uncertain whether the abrupt movement was the type II behavior described by Hamburger and Oppenheim (1967), video records were reexamined and all movements lasting <10 s also were identified. Abrupt movements were characterized by rapid joint excursions into either
flexion or extension and usually were followed by an abrupt return toward initial posture, as observed during the sequence in Fig. 3. The number of abrupt movements within motility pauses ranged from 0 to 25 occurrences per embryo at E12 (11.0 ± 6.8). Similar brief movements were found at E9 but with less frequency (P < 0.03), ranging from 1 to 8 occurrences per embryo (4.5 ± 3).

General kinematic features of motility sequences at E12

Time-position plots of joint excursions in the right wing and leg were constructed for 39 motility sequences at E12 totaling 2,168 s of kinematic data. E9 data for age-related comparisons consisted of 40 samples totaling 1,719 s. Kinematic samples were drawn equally from all embryos (5 sequences per embryo) except from one E12 embryo, because videotape damage prohibited computer acquisition of a fifth sequence. All kinematic samples at E12 exhibited features characteristic of motility at E9 (Fig. 4A). For example, motility sequences began with nearly synchronous onset of excursions in all joints of the wing and leg, and all joints typically returned to the initial premovement posture within a few seconds of one another at the end of the sequence (Fig. 4B). Second, over the duration of the sequence, the spatiotemporal pattern of elbow excursions was similar to that of the shoulder, and excursions at the ankle, knee, and hip were similar to one another (i.e., intralimb coordination). Third, for some part of each record, the spatiotemporal pattern of wing and leg excursions were similar to one another (i.e., interlimb coordination; Fig. 4, A and B), though this feature was less readily apparent in some samples at E12 (Fig. 5).

Select features typically distinguished E12 from E9 kinematic samples. Excursions at E12 appeared larger in amplitude and less regular, both spatially and temporally, with relatively abrupt excursions interposed between slow joint rotations (Figs. 3 and 4B). Joint excursions at E9, in contrast, tended to appear more regular, both spatially and temporally, across the majority of the sequence, followed by a short pause and ending with one to two brief excursions (Figs. 2 and 4A). Quantitative comparisons of the spatiotemporal features for E9 and E12 kinematic samples are presented in the following text.

Age-related changes in cycle parameters

Several, but not all, cycle parameters varied significantly with age. E12 embryos generated an average of 6.2–25.8 cycles per activity sequence (15.2 ± 4.9 elbow cycles; 12.5 ± 6.1 ankle cycles). E9 embryos produced an average of 8.6–16.0 cycles per sequence (elbow, 10.7 ± 1.7; ankle, 11.2 ± 2.1). Two-way ANOVA comparisons (age × joint) indicated age-related differences fell short of significant (P < 0.06).

Cycle duration averages and ranges for elbow and ankle excursions were significantly greater at E12 than at E9. Cycle duration averages and ranges for elbow and ankle excursions were significantly greater at E12 than at E9.
duration typically varied across consecutive cycles in a motility sequence at both ages; however, the temporal variability was substantially greater at E12 (Fig. 6). In contrast, temporal variability was less during the first four to six cycles of sequences at E9 with cycle durations of 2–4 s (see also Figs. 1B and 4A). Cycle duration data are summarized and the significant findings from two-way ANOVA comparisons (age x joint) are indicated in Table 1. On average, cycle duration at E12 was 300–1,100 ms longer and average within-subject variability 40–60% greater than at E9. The greater variability in cycle durations at E12 was partially attributable to age-related differences at both ends of the range; maximum cycle durations were 3–5 s longer and minimum cycle durations were 200 ms less than at E9. At both ages, the briefest cycles were observed at the ankle.

Parameters of coordination

Age-related transformations in kinematics included an increase in parametric estimates of intralimb coordination and a decrease in estimates of interlimb (ipsilateral wing/leg) coordination (Fig. 7). Two-way ANOVA comparison (age x joint) of correlation coefficients for concurrent joint excursions within a limb was significant for age indicating a greater percent of cycles closely covaried (r ≥ 0.7) at E12 than at E9 (P < 0.03). At E12, 61 ± 16% of elbow cycles and 51 ± 12% of ankle cycles yielded coefficients of r ≥ 0.7 (Fig. 7B). Where as at E9, 51 ± 17% of elbow cycles and 37 ± 12% of ankle cycles closely covaried (Fig. 7A). There was also a main effect for joint, indicating the percent of cycles yielding coefficients of r ≥ 0.7 was significantly greater for the elbow than the ankle (P < 0.03). Interlimb coordination was estimated by comparing concurrent excursions of the ankle and elbow, using the ankle cycle as reference for parceling the time series, and indicated that the extent of interlimb coordination was less at E12 than at E9. At E12, 18 ± 5% of cycles yielded a coefficient of r ≥ 0.7 (squares, Fig. 7B); whereas 26 ± 7% of cycles yielded a coefficient of r ≥ 0.7 at E9 (squares, Fig. 7A, P < 0.01).

The covariation between excursions of the elbow and ankle decreased from E9 to E12 despite the increased incidence of covariations within limb at E12. One possible explanation for reduced interlimb covariation at E12 is the greater difference in cycles closely covaried (Fig. 7A). There was also a main effect for joint, indicating the percent of cycles yielding coefficients of r ≥ 0.7 was significantly greater for the elbow than the ankle (P < 0.03). Interlimb coordination was estimated by comparing concurrent excursions of the ankle and elbow, using the ankle cycle as reference for parceling the time series, and indicated that the extent of interlimb coordination was less at E12 than at E9. At E12, 18 ± 5% of cycles yielded a coefficient of r ≥ 0.7 (squares, Fig. 7B); whereas 26 ± 7% of cycles yielded a coefficient of r ≥ 0.7 at E9 (squares, Fig. 7A, P < 0.01).

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TABLE 1. Averaged cycle duration parameters

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<th>Elbow</th>
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<td>Cycle duration, ms*</td>
<td>2,957 ± 583</td>
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<td>3,324 ± 869</td>
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<td>2,920 ± 540</td>
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<td>4,110 ± 1,002</td>
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<td>Within-subject standard, ms†</td>
<td>1,444 ± 389</td>
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<td>2,039 ± 603</td>
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<td>1,534 ± 392</td>
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<td>2,438 ± 392</td>
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<td>Maximum cycle duration, ms‡</td>
<td>9,873 ± 2,849</td>
<td>13,297 ± 3,365</td>
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<td>10,303 ± 2,440</td>
<td>15,019 ± 2,930</td>
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<td>Minimum cycle duration, ms§</td>
<td>550 ± 213</td>
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<td>317 ± 130</td>
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<td>424 ± 84</td>
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Values are means ± SD. *Age-related difference (P < 0.008). †Age-related difference (P < 0.001) ‡Age-related difference (P < 0.007) §Age-related difference (P < 0.0002) and elbow vs. ankle (P < 0.05)
elbow and ankle cycle period at E12 (786 ms) compared with the difference between joints at E9 (37 ms) summarized in Table 1. As correlations are sensitive to the range of values compared, it is also possible that the reduction in elbow/ankle covariation is partially attributable to an increased discrepancy in joint excursion ranges from E9 and E12. On average, the difference in elbow and ankle maximum range doubled from E9 (10.6°) to E12 (21°). The discrepancy in ranges is not likely a significant contributor to the reduction in covariations between limbs at E12, however, because excursion range increased significantly at all joints from E9 to E12 (Table 2).

A feature that readily distinguished E12 motility sequences from E9 sequences was the increased presence of abrupt joint excursions. At least two to three abrupt movements were present in all E12 sequences digitized. In some instances they were only apparent in leg excursions (Fig. 4B, *), but it was also common to find nearly synchronous abrupt excursions in the wing and leg (Fig. 3). In most instances, the excursions were abrupt in both directions, with the reversal in motion returning the joint to initial position (leg, Fig. 3); in other instances the reversal in motion exceeded initial position (wing, Fig. 3, **). In most E12 sequences, abrupt excursions were interposed between slower movements, but occasionally a series of abrupt excursions was observed (Fig. 3). Abrupt excursions were also apparent at the initiation and/or during the final one to two cycles of some E9 sequences (Fig. 1B). To further characterize and compare abrupt excursions, maximum excursion velocity for each joint was averaged within and across embryos. Two-way ANOVAs (with replication) indicated that maximum average maximum velocity was significantly greater at E12 across all joints during both excursions into extension and flexion (Table 3). There was also a significant increase in average velocity across all joints for both extension (P < 0.0001) and flexion (P < 0.0001) from E9 to E12. Despite differences in velocity and cycle period, comparisons of FFT analyses were not compelling, perhaps due to the greater variability in parameters at E12. Time series plots for E12 embryos contained a slightly greater portion of signal <0.5 Hz (P < 0.03); however, E9 embryos contained a slightly greater portion of signal <1.0 Hz (P < 0.05).

DISCUSSION

The boundaries drawn by the definitions of type I, II, and III motility continue to be useful for approaching the study of motility >30 yr after they were conceived originally (Hamburger and Oppenheim 1967). New technologies offer the opportunity to extend these definitions and examine more closely the nuances of motility for further probing mechanisms generating each behavioral form. The findings presented here reveal several kinematic attributes of type I and II motility and suggest some modifications in boundaries drawn by the original definitions that may be useful for future studies.

Emergence of type II motility

At the onset of this study, it was hypothesized that parameters of motility in E12 embryos would be sufficiently different from those at E9 to demonstrate a distinctive, second behavior (type II) was added to the embryo’s repertoire at E11. The definition of type II motility, i.e., sudden, rapid wriggles and startles of the whole body (Hamburger and Oppenheim 1967), appears to be consistent with the abrupt joint rotations noted in the time-position plots, suggesting these abrupt excursions are the kinematic correlates of type II motility. If this is correct, nearly all sequences at E12 contained samples of type II motility. In a few instances, abrupt movements characterized the majority of the motility sequence at E12 (Fig. 3), a kinematic pattern never observed in earlier studies of E9 embryos (Bradley 1997; Chambers et al. 1995). More typically, E12 motility sequences contained only two or three abrupt movements (Fig. 4B); however, as reported previously (Bradley 1997; Chambers et al. 1995), this pattern also was found in sequences at E9 (Fig. 1B). Thus if these abrupt excursions are type II motility, the findings indicate that it is not a newly added behavior at E11. Given the abrupt movements at E9 were significantly less frequent, typically small in amplitude and often limited to only one or two joints (i.e., Fig. 4A), it is possible direct observation methods used in earlier studies were insufficient for detecting type II behavior before E11.

Kinematic correlates of type I motility

One of the most striking features of kinematic data at E9 is the almost signature-like consistency found in time-position plots. One, motility sequences began with abrupt, nearly synchronous onset of joint excursions in the ipsilateral wing and

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<th>TABLE 2. Averaged maximum joint excursion range</th>
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<td>E9*</td>
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Values are means ± SD in degrees. *Age-related difference (P < 0.0001).

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<th>TABLE 3. Averaged maximum joint excursion velocity</th>
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<td>E9</td>
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Values are means ± SD in degrees per second. *Shoulder excursions into flexion were included in comparisons for extension motions at all other joints, and shoulder excursions into extension were included in comparisons for flexion motions at all other joints. Refer to Fig. 1A and text for details. †Age-related difference (P < 0.0001).
leg, and in the latter portion of the sequence, after a pause and one to two abrupt excursions, movement ceased nearly synchronously in all joints (Figs. 1B and 4A). Two, over the majority of the sequence, changes in joint position within a limb (i.e., shoulder and elbow) closely covaried as they alternately flexed and extended. Three, excursions of the homolateral wing and leg (i.e., elbow and ankle) also covaried but to a lesser extent than excursions within a limb. In earlier kinematic studies of motility (Bradley 1997; Chambers et al. 1995), the entire sequence was believed to be type I because the work of Hamburger and Oppenheim (1967) indicated it was the only behavioral form before E11.

Several of the signature-like features of motility at E9 summarized above also were present in kinematic samples at E12. Excursions of the wing and leg began nearly synchronously at the onset of nearly all sequences; there was typically a pause in all joints prior to the last one to two cycles; and excursions of all joints ceased nearly simultaneously at the end of most sequences. The general patterns of limb coordination at E12 were also similar to those at E9 with only modest changes in frequency of occurrence; the frequency of close covariations for joint excursions within a limb increased and the frequency of close covariation between homolateral limbs decreased with age. These general patterns persisted despite age-related increases in joint excursion range, excursion velocity, cycle duration range, and variability in timing of consecutive cycles. Thus given the consistency of the general pattern for motility sequences in the presence of age-related changes, it is proposed that the signature-like features described above (points 1–3), omitting abrupt movements, are the kinematic correlates of type I motility. This kinematic description of type I motility is viable because it applies at two different ages and is consistent with the original observational studies indicating that type I motility is the basic motility pattern of the chick embryo between E6 and E20 (Hamburger and Oppenheim 1967). Further, the presence of a general pattern in kinematic recordings is not necessarily at odds with the original definition of type I motility for the definition was formulated in reference to global patterns of movement, not the nuances of intra- and interlimb joint excursion patterns as emphasized by kinematic methods. Additionally, Hamburger and Oppenheim (1967) suggested that finer analytic methods might reveal patterns of coordination not readily detectable by direct observation before onset of type III motility, and a subsequent observational study provided general evidence of temporal correspondence in movements of ipsilateral limbs over the embryonic period (Provine 1978).

Transformations in temporal structure of type I motility

The most compelling transformation in motility sequences between E9 and E12 was the apparent disintegration of the underlying rhythmicity that typifies initial cycles of an activity sequence at E9 (Chambers et al. 1995). At E9 there is general consistency in the cycle duration of consecutive joint rotations in the initial four or more cycles of a sequence (Figs. 4A and 6). The duration of consecutive limb excursions at E9 is consistent with cycle durations of 3–4 s for EMG recordings from E9 embryos in ovo (Bradley and Bekoff 1990). This temporal pattern is also similar to cycle durations observed in neurogram recordings during repetitive motor neuron activity after surgical reduction at E9 (Ho and O’Donovan 1993; O’Donovan and Landmesser 1987). However, comparison of data for intact embryos (i.e., Figs. 1B and 4A) and surgically reduced embryos (O’Donovan and Landmesser 1987, Fig. 3C; Ho and O’Donovan 1993, Fig. 12A) gives the impression that the rhythmicity is more stereotypic and sustained for a greater number of cycles after surgical reduction. The temporal pattern observed in E9 intact embryos was not observed in E12 intact embryos. Although records for E12 intact embryos exhibited some temporal structure (e.g., Figs. 3 and 4B), cycle duration across consecutive cycles was more irregular (Fig. 6) and average deviation in cycle duration was significantly greater than in E9 intact embryos. Emerging differences between E12 and E9 embryos may be a function of descending and peripheral input strength, as both undergo significant elaboration between E9 and E12 (see INTRODUCTION).

The temporal stability of movement cycles during type I motility at E9 appears to be attributable to the unique physiology of immature spinal neurons. O’Donovan and colleagues recently proposed that the underlying rhythmogenesis in young embryos is an emergent property of transient, population-dependent dynamics among ventral neurons due to recurrent connectivity (O’Donovan and Chub 1997) rather than the expression of an immature pattern generator for locomotion (Chub and O’Donovan 1998). During early embryonic development, activity-dependent depression observed at 1α afferent synapses on motor neurons (Lee and O’Donovan 1991) may fine tune burst frequency within a motility sequence and subsequent hyperpolarization may terminate the sequence (O’Donovan and Chub 1997; O’Donovan and Rinzell 1997). The temporal stability of movement cycles at E9 also may be a consequence of excitation unique to GABAₐ-mediated activation of voltage-gated cation channels and/or N-methyl-D-aspartate (NMDA) receptors and the slow decay of NMDA currents during early development, as observed in the hippocampus (Ben-Ari et al. 1997). In support of this view, rhythmic activity of spinal neurons is blocked by application of bicuculline, a GABAₐ antagonist (O’Donovan and Chub 1997). Several lines of evidence suggest that NMDA currents contribute to rhythmic motility. NMDA antagonists transiently block rhythmic activity (Barry and O’Donovan 1987); the number of cycles per sequence is increased after bath-application of NMDA (Ho and O’Donovan 1993; O’Donovan and Landmesser 1987); and fluctuations in calcium currents are coincident with rhythmic ventral root activity (O’Donovan et al. 1994). In more recent work, Chub and O’Donovan (1998) provide evidence that there may be more than one state capable of producing spontaneous rhythmicity in the embryonic spinal network, one dependent on glutamate/cholinergic connections and another on GABA/glycinergic connections. Thus age-related changes in one or both states may underlie age-related changes in the temporal structure of type I behavior.

The mechanisms responsible for the temporal transformations in type I motility observed in intact embryos between E9 and E12 are not immediately known but may be related to the progressive decline in motility after peak activity at E15 (Hamburger et al. 1965). The loss of temporal stability within a motility sequence at E12 might be a function of increasing GABA-mediated inhibition, as GABA has been observed to diminish the strength of immature AMPA-mediated NMDA currents in neonatal hippocampal CA3 neurons (Ben-Ari et al.
Transformations as early preparations for hatching

Even in earliest descriptions of motility, Hamburger saw purpose in the embryo’s movements, suggesting they were critical to differentiation of the skeleton and muscles (Hamburger 1963; see also Hall and Oppenheim 1987). Transformations between E9 and E12, increased excursion range, excursion velocity, cycle period range and variability, increased intralimb coordination, and decreased interlimb coordination also may have functional significance. For example, these transformations may be prerequisites for the adaptive, coordinated behaviors of walking, running, scratching, and foot shaking seen within hours after hatching. The increasing strength of intralimb coordination and greater movement velocities associated with abrupt movements may be prerequisite refinements for producing large propulsive forces at the onset of hatching. The increasing intralimb coordination also may be preparatory for the greater running than walking abilities of new hatchlings (Muir et al. 1996), when ability to stay with a mobile brood is more critical than energy efficiency. The decreasing interlimb coordination, or increasing disassociation, of wing and leg movements is likely to be preparatory for differential use of the wings (for flapping) and legs (for locomotion) after hatching.

In sum, evidence from a broad array of studies demonstrates that there is considerable organization of spinal circuits supporting rhythmogenesis by E9. Current results extend those findings indicating the embryonic circuits produce reliable and orderly movement elements definitive of type I motility. Most of these movement elements are still evident during motility at E12 as spinal circuit remodeling alters temporal attributes of rhythmogenesis. The increasing variability observed in type I motility between E9 and E12 is likely indicative of maturational changes in spinal networks as afferent and descending influences mold motor patterns in preparation for hatching and posthatching behaviors. Finally, the data suggest that type II motility is expressed concurrently with type I motility at an age earlier than previously recognized. Collectively, these findings suggest that even earliest movements are organized behaviors, rather than random and uncoordinated events, that may have purpose and with further refinement may be fundamental elements of adaptive behaviors after hatching.

Special thanks to J. Bowers, J. Fairley, D. Han, C. Kostic, M. Painter, D. Parks, and C. Sebelski for assistance in data processing. This work was funded by National Science Foundation Grant IBN-9421125 and the Zambeze Research and Innovation Fund, University of Southern California.

Address for reprint requests: Dept. of Biokinesiology and Physical Therapy, University of Southern California, 1540 E. Alcazar St., CHP155, Los Angeles, CA 90033.

Received 1 June 1998; accepted in final form 9 December 1998.

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