

Development of Interlimb Movement Synchrony in the Rat Fetus

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In the fetal rat, interlimb synchrony is a prominent form of temporally organized spontaneous motor activity in which movement of different limbs occurs at nearly the same instant. In the present study, synchrony profiles were created for different pairwise combinations of limbs over the last 5 days of gestation. Observed rates of synchrony differentiated from randomized time series from Gestational Day 19 to Day 21 (E19–E21), with forelimb synchrony emerging earlier than that of other limb pairs. Synchrony profiles were elevated at the shortest intervals between successive limb movements, indicating that movements became more tightly coupled toward the end of gestation. Interlimb synchrony appears to be a robust method of quantifying fetal movement and may prove useful as a tool for assessing prenatal nervous system functioning.

The knowledge that vertebrate embryos move prior to birth has spawned a long-standing interest in how quantification of this behavior might improve our understanding of nervous system functioning and development. This early motor behavior has been characterized as twitchlike (Hall & Oppenheim, 1987; Hamburger, 1963), and although they appear random or unorganized to the casual observer, researchers have found these early movements to show both spatial and temporal organization (de Vries, Visser, Mulder, & Prechtel, 1987; Robertson, 1987; Robertson & Bacher, 1995; Robinson & Smotherman, 1988, 1992a). Changes in the patterns of embryonic movement that correlate with development of the nervous system have also been found (Bekoff, 1995; Hamburger, Wenger, & Oppenheim, 1966; Ripley & Provine, 1972). Further, these movements require no exogenous stimulation, suggesting that they are the spontaneous integrated output of a developing nervous system (Hamburger et al., 1966; Landmesser & O'Donovan, 1984).

Because of this close relationship between the development of the underlying nervous system and changes in the patterns of spontaneous movement prior to birth, the question has often been raised as to whether such measures of behavior might be useful in assessing the status of nervous system development (Prechtel & Einspieler, 1997). Simple quantitative approaches to the assessment of fetal movement have concentrated on gross body movements, which typically have not yielded robust or meaningful results (D'Elia, Pighetti, Moccia, & Di Meo, 1998; DiPietro et al.,

2002; Nijhuis et al., 1999). However, other investigators have emphasized the temporal aspects of movements among various body parts as a more useful indicator of nervous system functioning. These approaches to quantifying early motor behavior have provided a clear picture of developmental trends across a number of species, including the chick embryo (Bekoff, 1995; Provine, 1980), rodent fetus (Kleven & Robinson, 2000; Kodama & Sekiguchi, 1984; Robinson & Smotherman, 1987, 1988, 1992b, 1992c), sheep fetus (Natale, Clewlow, & Dawes, 1981; Robertson & Bacher, 1995), rat infant (Robinson, Blumberg, Lane, & Kreber, 2000), and human infant (Key et al., 2001; Kisilevsky & Low, 1998; Kleven, Key, Lane, Lauer, & Robinson, 2001; Thelen, 1979, 1985). Additionally, these measures are robust across a wide range of temporal dimensions (de Vries et al., 1987; Robertson, 1987; Robertson & Bacher, 1995; Robinson & Smotherman, 1988, 1992a).

Further refinement, by limiting quantification to limb movements, has brought even greater clarity to these temporal relationships. One such measure is the quantification of multilimb bout structures. A bout may be defined as the successive occurrence of two or more behavioral events within a specified period of time (Fagen & Young, 1978; Machlis, 1977). When a criterion of 0.2 s or less between successive limb movements is used to define a multilimb bout, clear continuities emerge across both fetal and postnatal ages of rat development (Robinson et al., 2000). Additionally, a simple computational model of these age-dependent results suggests that only spinal cord circuitry is necessary to create these movement patterns. This prediction was supported experimentally by the finding that early movements are changed little by high cervical transection, which isolates the spinal cord from the brain (Robinson et al., 2000). However, midthoracic spinal transection has been shown to reduce hindlimb movements by 40%–50% in both fetal and infant rats (Blumberg & Lucas, 1994; Robertson & Smotherman, 1990). Taken together, these computational and experimental models of the effects of cord transection on fetal movement demonstrate that descending influences from the brain play little or no role in generating these early behavioral patterns, but communication among various segments of the spinal cord is necessary to produce the exact patterns of

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Behavioral analyses reported in this study were conducted from data that contributed to different analyses in a previously published report (Robinson et al., 2000). Additionally, preliminary findings of the results reported herein have been presented at a meeting of the International Society for Developmental Psychobiology (Lane & Robinson, 1998) and the International Conference on Infant Studies (Stansfield & Robinson, 1998). This research was supported by National Institute of Child Health and Human Development Grant HD 33862 to Scott R. Robinson.

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spontaneous movement observed during early perinatal development (Robinson et al., 2000).

Communication among spinal cord segments is also necessary for specialized functions, like locomotion, observed in adult animals. Many mammalian species use limb pairs in varying combinations for different specialized tasks that require quite different coordination between or within spinal segments. For example, the laboratory rat uses all four limbs for locomotion, requiring coordination among all its limbs across many segments of the spinal cord. However, the rat also uses its forelimbs for an assortment of highly specialized grooming and food manipulation tasks that require good neuronal coordination within the forelimb girdle and corresponding spinal segments. While the rat is performing these forelimb movements, the hindlimbs are differentially used for postural support, which would necessitate an entirely different type of coordination within the hindlimb girdle segments than would be seen in locomotion. However, these roles change significantly when the rodent scratches with one of its hindlimbs, requiring the other three limbs for postural support and necessitating yet another form of coordination both between and within various spinal segments.

Whereas the coordination of movement between limbs in an adult animal varies with specialized behavior and appears highly stereotyped to the casual observer, behavioral embryologists have noted a rich epigenetic emergence of these various motor patterns well before birth (Gottlieb, 1997; Hall & Oppenheim, 1987; Oppenheim, 1982; Robinson & Smotherman, 1991, 1992b, 1992c). This prenatal emergence of organized behavior, which is not limited to locomotor patterns, requires varying coordination both between and within various limb girdles and their corresponding spinal segments. Temporal coordination among limbs during prenatal development has previously been characterized as multilimb bouts by researchers using probability risk plots to quantify temporal dependencies among limbs without respect to specific limb combinations (Robinson et al., 2000).

To further investigate the developmental origins of interlimb coordination that may underlie specialized limb behavior, we conducted a detailed analysis of the early temporal relationships between various limb combinations. In this study, interlimb synchrony was used to quantify the temporal relationship between pairwise combinations of limbs across the last 5 days of prenatal development in the rat. Movement of two different limbs may be considered synchronous if it occurs nearly simultaneously, that is, if the two movements are initiated within 1 s of each other (Robinson & Smotherman, 1987). In the present study, the analysis of interlimb synchrony used a time series of fetal movements scored from video recordings, which enables more precise quantification of limb movement events (accurate to 0.1 s). This degree of resolution allows the developmental pattern of temporal relationships in a limb pair to be characterized more fully. The degree of temporal coupling was measured between forelimbs, hindlimbs, ipsilateral limbs (same side forelimb and hindlimb), and contralateral limbs (diagonal forelimb and hindlimb) in prenatal rats from Gestational Day 17 to Day 21 (E17–E21). These data were then used to construct profiles of interlimb synchrony that reveal systematic changes in this form of motor coordination during prenatal development.

Method

Archival Data

Subjects. Subject fetuses were the offspring of Sprague-Dawley Norway rats (*Rattus norvegicus*). During a 4-day breeding period, adults were housed together (3 females with 1 male) in polycarbonate cages with hardwood bedding and provided with food and water ad libitum. Cages were kept in colony rooms where temperature and humidity were controlled, and a 12-hr light–dark regime was maintained. Date of conception (E0) was determined by positive vaginal smears, which were collected daily over the 4-day breeding period. These time-mated females remained housed together until testing at one of five gestational ages (E17–E21). At all times, both adult and fetal subjects were cared for in accordance with animal care guidelines established by the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, 1986).

Preparation of subjects. Forty pregnant females (8 at each of the five gestational ages, E17–E21) were prepared for testing, under brief ether anesthesia, with an injection of 100% ethanol into the spinal canal between the first and second lumbar vertebrae. This chemomyelotomy procedure produces irreversible spinal anesthesia, eliminating sensation in the lower body of the female. After spinal preparation, the female was secured in a Plexiglas holding device and immersed to chest level in a buffered isotonic saline bath (Locke's solution) maintained at 37.5 ± 0.5 °C. The uterus then was externalized into the bath through a midline laparotomy, and the female was left undisturbed for 20 min to ensure that both mother and fetus had fully recovered from the ether anesthesia.

After acclimation, individual fetuses were externalized from the uterus into the saline bath, and their embryonic membranes, both chorion and amnion, were removed to facilitate visibility of all four limbs. Additionally, fetal subjects were secured in a prone position on a holding apparatus with a band across the thorax. Fetal attachment to the placenta and uterus by the umbilical cord was maintained, and the preparation was monitored throughout the testing period for signs of deterioration or distress. This experimental procedure for externalization and subsequent observation of fetal rodents has been extensively used (Smotherman & Robinson, 1991).

Procedure for scoring behavioral data. Spontaneous motor activity of fetal subjects was videotaped during either a 15-min (E17 fetuses) or a 30-min (E18–E21) session. These ages were chosen to include a range from just after inception of movement (E16) until the day before birth. An S-VHS format at SP recording speed with simultaneous SMPTE time code generation was used during taping, which provided high-quality recordings and precise synchronization of individual video frames during playback.

The resulting video records comprised 18 hr of fetal behavioral data. Fetal limb movements were scored, one limb at a time, from the entire video session during normal-speed playback using an event recorder accurate to 0.1 s. For each limb of a subject, the tape was cued to the same video frame to ensure synchronization of the event records. A movement event was defined as any active movement of the limb. No differentiation was made between gross or fine limb movements or among the duration, direction, or magnitude of movement or pattern of coordination among limbs.

For assessment of scoring reliability, single limbs were scored multiple times from sections of video not used in this study. The multiple passes were then compared for correlation. A reliability correlation of at least .90 was achieved for both forelimbs and hindlimbs before scoring the selected tape sections. This process was repeated at each gestational age, because of the differences in movement of fetuses at various ages.

Data Analysis

After scoring, the event files were prepared for data analysis with computer software written by the author. First, scored files for the individual limbs were interleaved to produce six pairwise combinations: fore-

limbs, hindlimbs, right ipsilateral limbs (right fore- and hindlimb), left ipsilateral limbs (left fore- and hindlimb), and two contralateral limb combinations (right forelimb with left hindlimb and left forelimb with right hindlimb). The time lag between successive movements of different limbs was rounded to the nearest 0.1 s; these temporal bins are hereafter referred to as intervals. The number of synchronous limb movements was then counted within intervals ranging from 0.0 to 1.2 s. Preliminary analyses indicated that synchrony profiles declined to levels that did not differ from random by 0.6 s. To ensure that all analyses incorporated the entire period of elevated synchrony, we included measurements for twice this duration (i.e., 1.2 s). These frequencies were subsequently divided by the time that both limbs were observed to obtain synchrony rates per minute for each of the 13 intervals.

To aid in determining if the resulting rates of synchrony occurred by chance, we wrote Monte Carlo software to randomize the data. The prepared pairwise data files were first separated into individual limb files. The events in these files were then shuffled, preserving both the number of visible limb movements in each file and the intervals between movements but varying the sequential order of those intervals. This process of randomization and shuffling, which preserves both the activity level and patterning of each subject's movements, was repeated 10 times. The resulting 10 sets of files were re-interleaved and processed in the same manner as the original data files. Finally, the 10 sets of synchrony interval rates were averaged to produce Monte Carlo mean synchrony rates per minute at each of the 13 intervals.

The resulting synchrony profiles from both the observed and Monte Carlo (mean randomized) data for each subject were analyzed in a series of Kolmogorov-Smirnov (K-S) two-sample tests (Siegel & Castellan, 1988). The K-S test, which has been used in the analysis of movement cyclicality in infants and fetuses (Robertson, 1987; Robertson & Smotherman, 1990), permits the comparison of temporal patterning between entire profiles of observed and Monte Carlo synchrony rates. Further, this test allowed us to determine whether movements are different from random on a subject-by-subject basis. An alpha of $p < .05$ was set for all K-S tests.

The resulting synchrony profiles were analyzed separately in a series of three-factor Age \times Interval \times O/MC (Observed vs. Monte Carlo) analyses of variance (ANOVAs). The interval and O/MC factors were treated as repeated measures, because the same subjects contributed data to all the interval levels and both the observed and Monte Carlo treatment conditions. The two ipsilateral limb combinations were collapsed into a single dataset consisting of mean rates for the two limb pairs. Likewise, the two contralateral limb combinations were merged by calculating the mean synchrony rates of the right forelimb-left hindlimb and left forelimb-right hindlimb pairs. Separate analyses were run for the four resulting limb combinations: forelimbs, hindlimbs, ipsilateral limbs, and contralateral limbs. To aid in the interpretation of significant interactions, we performed ANOVA tests of simple main effects of age at each interval (observed data only), and observed versus Monte Carlo intervals at each age. Because this entailed a large number of statistical tests, a more conservative alpha level of $p < .01$ was used to judge significance in these analyses. Following significant simple main effects, post hoc comparison of mean synchrony rates was performed using Fisher's protected least squares difference.

In addition to actual synchrony rates, relative rates of synchrony were also calculated for the forelimb and hindlimb combinations. Relative synchrony was calculated as the frequency of synchronous movements of a limb pair divided by the average of all movements of the same two limbs. These synchrony profiles then were analyzed in two 2-way ANOVAs, with age as a between-subjects factor and synchrony interval as the within-subject factor. Further, comparisons of synchrony profiles from the various 5-min segments of data were explored to determine whether synchrony profiles were stable for each individual or varied over the 30-min observation period. To explore these possible activity-dependent changes in synchrony profiles, comparisons were made of synchrony profiles selected from the two 5-min periods of lowest and highest activity for each subject.

Means of these maximum and minimum synchrony profiles were then analyzed in two (forelimbs, hindlimbs) 3-factor ANOVAs with maximum versus minimum (max/min) and synchrony interval as repeated measures. Unless otherwise stated, an alpha of $p < .05$ was set for all statistical tests, which were run using StatView software Version 5 (SAS Institute Inc., 1998).

Results

Movement

Figure 1 shows the mean rates per minute of individual fetal limb movements across the observed ages. An overall two-factor ANOVA to compare changes in forelimb and hindlimb activity at the five ages showed the significant interaction between limb and age, $F(4, 70) = 3.3$, $p = .02$. To simplify this interaction, we calculated two 1-way ANOVAs to compare forelimb or hindlimb movements across age. The analysis of forelimb activity revealed no difference in movement rates across ages, $F(4, 35) = 1.1$, $p = .39$. However, hindlimb activity did increase across ages, $F(4, 35) = 8.1$, $p < .01$. E17 hindlimb movements were significantly reduced compared with all later ages (E18-E21), and E18 hindlimb movements were significantly lower than E20 and E21. This pattern is consistent with a monotonic increase in hindlimb activity from E17 to E20. In a post hoc pairwise analysis of left and right hindlimbs at each age, no difference was found between the two hindlimbs at any age.

Monte Carlo Comparisons

To determine whether synchronous movements between limb pairs occurred at better than chance rates, a two-sample K-S test was run to compare the observed and Monte Carlo (mean randomized) synchrony profiles for each subject. Separate tests were run for each of the four limb combinations: forelimbs, hindlimbs, ipsilateral limbs, and contralateral limbs. Figure 2 (top) shows representative forelimb profiles of mean synchrony for an E17 and an E20 subject, along with both the mean Monte Carlo and each of the individual randomizations.

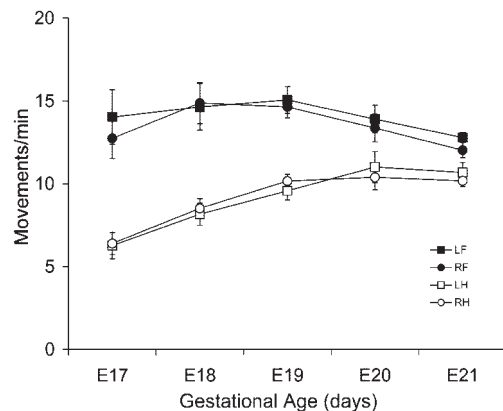


Figure 1. Developmental profiles of movement rates for each of the four limbs: left forelimb (LF), right forelimb (RF), left hindlimb (LH), and right hindlimb (RH). Points show the mean number of movements per minute at each of the gestational ages observed. Error bars depict the standard error of the mean.

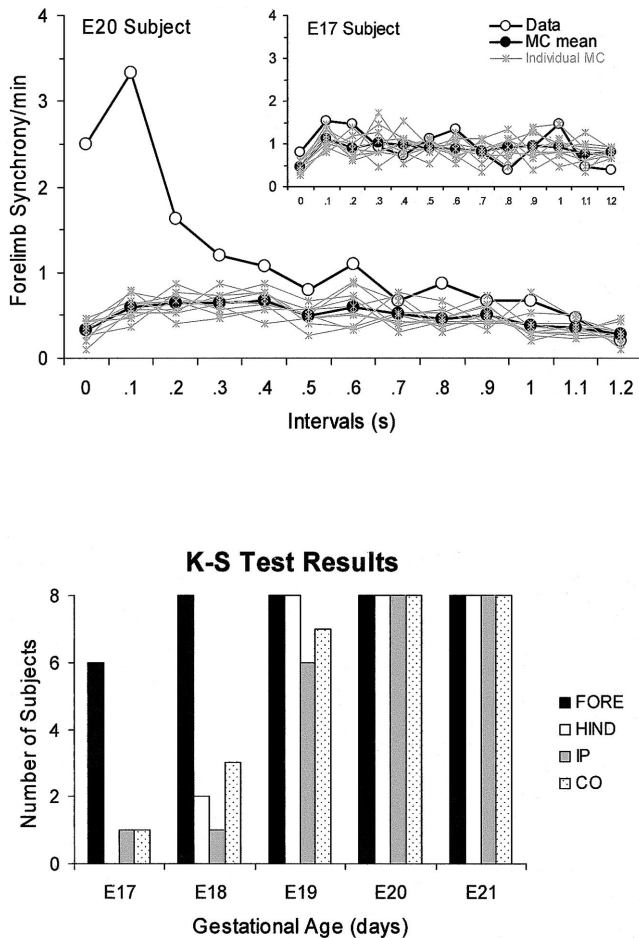


Figure 2. Top: Interlimb movement synchrony profiles for individual fetal subjects. The larger panel depicts forelimb synchrony for a subject on Gestational Day 20 (E20) whose observed synchrony profile was found to be significantly different from a profile produced by the Monte Carlo randomization process. The smaller inset shows forelimb synchrony for a subject on E17 that does not differ from randomized data. Points show the frequency of synchronous movements per minute at each of the thirteen 0.1-s intervals for the observed data (Data), the means of the Monte Carlo randomized data (MC mean), and the individual Monte Carlo randomizations (Individual MC). Bottom: Results of the Kolmogorov–Smirnov (K-S) test comparing the observed and randomized data for each subject. Bars depict the number of subjects at each age whose profiles differed from random for the forelimbs (FORE), hindlimbs (HIND), ipsilateral (IP) limb pairs, and contralateral (CO) limb pairs.

In Figure 2 (bottom), the K-S test results show an increase across ages in the number of subjects whose observed profiles differed significantly from the randomized data. This pattern of age-related change is consistent for all four pairwise limb combinations, with the forelimb pairs different in all subjects by E18, followed by the hindlimbs on E19, and culminating in all subjects' limb pairs significantly different from their randomized data on the last 2 days of gestation.

Interlimb Movement Synchrony

Frequency by 0.1-s intervals. We conducted an initial overall ANOVA for each limb combination to compare the effects of age,

O/MC, and interval on rates of interlimb synchrony. Table 1 shows the results from these overall ANOVAs. Significant two- and three-way interactions involving all factors were seen for the profiles of all four limb combinations. For each age at which most subjects showed overall observed synchrony profiles that differed from the Monte Carlo profiles for all limb pairs, follow-up ANOVAs for the simple main effect of observed versus Monte Carlo were conducted at each interval (i.e., E17 and E18 were excluded). These tests revealed significant differences ($p < .01$) between the observed and Monte Carlo data for intervals greater than 0.5 s in only 6 of 84 comparisons (7.1%). This contrasts sharply with intervals of 0.5 s or less, in which 61 of 72 comparisons (84.7%) were significant. Therefore, the remainder of the analyses are reported only for observed data and those shorter intervals of 0.5 s or less.

For the forelimb pair, a simple main effect of age at each interval (observed data only) was observed for the intervals of 0.0 s, $F(4, 35) = 5.7, p < .01$, and 0.1 s, $F(4, 35) = 6.1, p < .01$. Visual inspection of the profiles (see Figure 3, top) revealed tight coupling of the forelimbs for all ages, as evidenced by higher rates of synchrony at 0.1 s, relative to other intervals. Additionally, a developmental trend was seen with a dramatic increase in synchrony after E17 that persisted across gestation. Follow-up tests confirmed this trend, with significant differences between the earliest two ages (E17 and E18) and all later ages, at the 0.0-s interval. A similar pattern was seen at the 0.1-s interval except that E18 was not significantly different from E21.

For the hindlimbs, a simple main effect of age at each interval was observed for all the lower intervals, as follows: 0.0 s, $F(4, 35) = 4.2, p < .01$; 0.1 s, $F(4, 35) = 9.1, p < .01$; 0.2 s, $F(4, 35) = 11.1, p < .01$; 0.3 s, $F(4, 35) = 4.5, p < .01$; 0.4 s, $F(4, 35) = 3.8, p < .05$; and 0.5 s, $F(4, 35) = 8.2, p < .01$. In contrast to the forelimbs, profiles of the hindlimbs (see Figure 3, bottom) showed little organized coupling on the first 2 days of observation (E17 and E18). On E19, however, higher rates of synchrony emerged at the 0.0-s through 0.2-s intervals, peaking in frequency at the tightly coupled interval of 0.1 s on the last 2 days of gestation. Post hoc tests confirmed significant differences at the 0.0-s interval between E17 and the later ages (E19–E21), as well as between E18 and the last 2 days before birth. Similarly, at both the 0.1-s and 0.2-s intervals, both E17 and E18 were significantly different from the later three ages, but E20 also was significantly elevated from E19 at the 0.2-s interval. Intervals 0.3–0.5 s also showed typical patterns, with E17, E18, and occasionally E19 significantly different from E20–E21.

As was seen in the hindlimb analysis, the between-girdle pairs (ipsilateral and contralateral limb combinations) revealed simple main effects of age at each interval for most of the intervals at 0.5 s and lower (see Figure 4). The results for the ipsilateral limb pairs were as follows: 0.0 s, $F(4, 35) = 5.6, p < .01$; 0.1 s, $F(4, 35) = 7.1, p < .01$; 0.2 s, $F(4, 35) = 11.8, p < .01$; 0.3 s, $F(4, 35) = 6.7, p < .01$; 0.4 s, $F(4, 35) = 5.5, p < .01$; and 0.5 s, $F(4, 35) = 3.0, p < .05$. Similarly, the contralateral limb combinations showed significance as follows: 0.0 s, $F(4, 35) = 13.3, p < .01$; 0.1 s, $F(4, 35) = 9.4, p < .01$; 0.2 s, $F(4, 35) = 16.0, p < .01$; 0.3 s, $F(4, 35) = 4.7, p < .01$; and 0.4 s, $F(4, 35) = 3.9, p < .05$. Another similarity to the hindlimbs was that the between-girdle synchrony profiles increased in frequency over the younger ages, peaking at

Table 1
Three-Way Analysis of Variance for Interlimb Synchrony

Source	dfs	F			
		Forelimbs	Hindlimbs	Ipsilateral	Contralateral
Between subjects					
Age (A)	4, 35	1.2	10.6	3.1*	5.0**
Within subjects					
Observed and Monte Carlo (O/MC)	1, 35	855.2**	853.6**	782.0**	486.7**
A × O/MC	4, 35	6.2**	16.0**	6.1**	8.3**
Interval (I)	12, 420	73.2**	63.2**	110.3**	100.1**
A × I	48, 420	4.0**	4.4**	7.5**	8.4**
O/MC × I	12, 420	56.7**	56.7**	79.6**	75.9**
A × O/MC × I	48, 420	3.9**	3.8**	6.2**	7.6**

* $p < .05$. ** $p < .01$.

E20 and E21. Post hoc tests confirmed this developmental trend for both between-girdle limb combinations. At the 0.0-s interval, significant differences were seen between E17 and E19–E21, and E18 and the last 2 days of gestation, for both between-girdle limb pairs. E19 also showed a significant difference from E20 and E21

in the contralateral pair. These patterns of significance generally were true of the 0.1-s and 0.2-s intervals as well, with E17 also different from E18. Finally, post hoc tests on the 0.3-s through 0.5-s intervals mainly revealed differences between E17 and later ages.

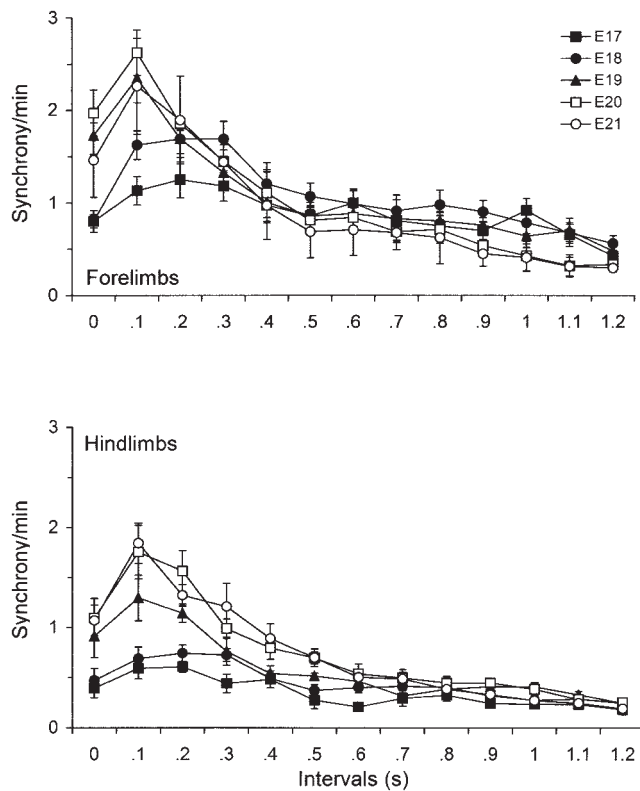


Figure 3. Developmental profiles of interlimb movement synchrony at each gestational age (E17–E21) for the forelimbs (top) and hindlimbs (bottom). Points show the mean number of synchronous movements per minute observed at each of the thirteen 0.1-s intervals. Error bars depict the standard error of the mean.

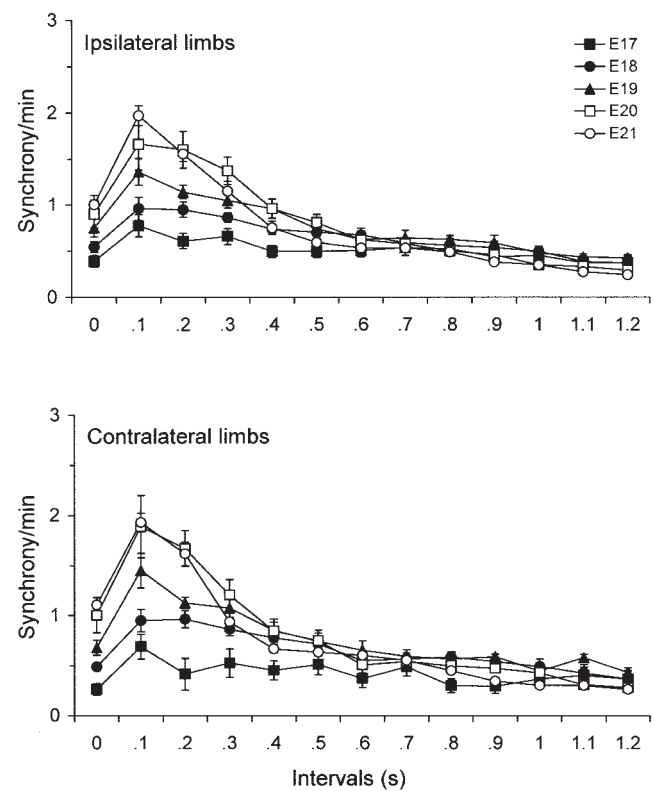


Figure 4. Developmental profiles of interlimb movement synchrony at each gestational age (E17–E21) for the ipsilateral (top) and contralateral (bottom) limb pairs. Points show the mean number of synchronous movements per minute observed at each of the thirteen 0.1-s intervals. Error bars depict the standard error of the mean.

Synchrony as a percentage of movement. To determine whether the developmental changes in synchrony profiles were the result of corresponding changes in rates of movement, we expressed synchrony profiles as a percentage of average limb pair movement. ANOVA results are shown in Table 2. Significant main effects and interactions of age with interval were seen for both forelimb and hindlimb pairs after standardizing by movement frequency. Figure 5 shows the mean synchrony profiles at each age for the two limb combinations. Both hindlimb and forelimb profiles showed the same developmental trends seen in the raw frequency graphs (see Figure 3). Specifically, E17 and E18 showed lowest rates of synchrony, with E19 at an intermediate level, and E20 and E21 at the highest rates. Simple main effects of age at each interval and pairwise comparisons confirmed the similarity of the distribution pattern across ages, in both the frequency and percentage of movement profiles. For both forelimbs and hindlimbs, simple main effects of age at each interval were found for most of the lower intervals (0.0 s through 0.2 s). Forelimb results were as follows: 0.0 s, $F(4, 35) = 4.6, p < .01$; 0.1 s, $F(4, 35) = 6.8, p < .01$; 0.2 s, $F(4, 35) = 4.0, p < .01$. Similarly, the hindlimbs showed a significant effect of age at the 0.1-s interval, $F(4, 35) = 6.8, p < .01$, and the 0.2-s interval, $F(4, 35) = 4.2, p < .01$. As was the case with the frequency data, pairwise tests revealed significant differences between the earlier ages (E17 and E18) and the later ages (E20 and E21), with E19 occasionally different from one or both of these two age groups.

Synchrony across 5-min time periods. We compared interlimb synchrony from 5-min periods of maximum and minimum activity rates for each individual to determine whether synchrony rates were stable across the observation period. Table 3 shows the results of a three-way ANOVA for synchrony profiles from the 5-min periods of maximum and minimum synchrony rates for each subject. No main effect or significant interaction was found for the max/min factor for either the forelimb or hindlimb pairs. Sample comparisons of mean maximum and minimum profiles for E17 and E20 are shown in Figure 6. As predicted by the overall ANOVA, there is little difference between profiles from minimum and maximum rates of synchrony.

Discussion

The results of this study describe and quantify the emergence of clear developmental patterns of interlimb synchrony in the spon-

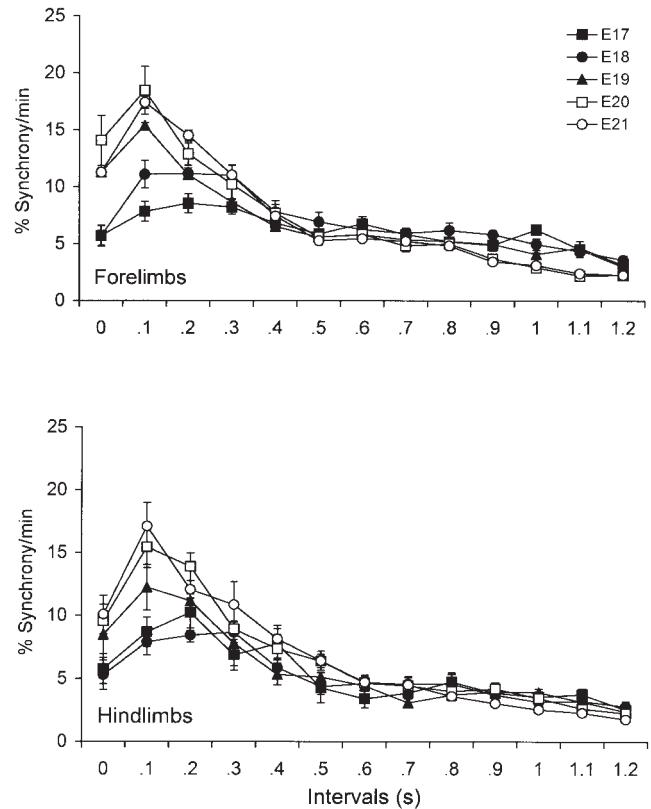


Figure 5. Same profiles of interlimb movement synchrony shown in Figure 3 but expressed as a percentage of movement in the forelimbs (top) and hindlimbs (bottom). Points show the mean percentage of synchronous movements per limb pair movement expressed as a rate per minute during observation, for each of the thirteen 0.1-s intervals. Error bars depict the standard error of the mean.

taneous movement of the fetus. Not only did various pairs of limbs show differential timing of emergence from random patterning, but the patterns of synchronous movement also differed across development. For example, the majority of subjects exhibited synchrony profiles of the forelimbs that were different from random even at the earliest observation time (E17), which is just 1 day after movement inception (the earliest age of movement). Despite this early emergence from random, it was not until 2 days later at E19 that the forelimbs showed the tightest coupling, as evidenced by a strong peak at 0.1 s. A similar pattern of emergence and change across development was seen in the hindlimbs as well as the two between-girdle pairs (ipsilateral and contralateral). However, these changes occurred a day later relative to the forelimbs.

Of particular interest are the specific changes in the profiles across development. Although all limb combinations showed relatively low levels of synchrony at all intervals of time at the earliest observations periods, they all also showed elevations across development. However, these elevations in synchrony were not distributed equally across the various intervals but significantly increased at the lower intervals of 0.0 s through 0.2 s. These changes in the shape of the profile distributions suggest that although increases in movement rates might be contributing to increases in interlimb movement synchrony, they do so differen-

Table 2
Two-Way Analysis of Variance for Synchrony Percentage of Movement

Source	dfs	F	
		Forelimbs	Hindlimbs
Between subjects			
Age (A)	4, 35	5.3**	12.0**
Within subjects			
Interval (I)	12, 420	63.6**	61.7**
A × I	48, 420	4.1**	2.5**

** $p < .01$.

Table 3
Three-Way Analysis of Variance for Maximum Versus Minimum Relative Synchrony

Source	dfs	F	
		Forelimbs	Hindlimbs
Between subjects			
Age (A)	4, 35	1.4	1.0
Within subjects			
Maximum/Minimum (Max/Min)	1, 35	0.9	1.0
A × Max/Min	4, 35	0.2	1.0
Interval (I)	12, 420	35.0**	35.0**
A × I	48, 420	2.5**	2.3**
Max/Min × I	12, 420	1.7	1.0
A × Max/Min × I	48, 420	0.9	1.0

** $p < .01$.

tially. Specifically, only those intervals of tight interlimb movement coupling (0.0 s–0.2 s) increased across development.

Closer inspection of the data revealed two additional findings that support the hypothesis that changes in interlimb synchrony were not driven by changes in rates of movement. First, forelimb movements did not change significantly across age (see Figure 1). However, the synchrony profiles for the forelimbs changed, not only in frequency, but also in the distribution of those frequencies across intervals, which was particularly evident in the height of the peak at an interval of 0.1 s (see Figure 3). In contrast, hindlimb movement rates did change, showing a significant increase from E17 to E20. However, synchrony rates that showed the most pronounced increase were those in the lower interval ranges, particularly at the 0.1-s interval. Even when standardized by movement rates (see Figure 5), the synchrony profiles continued to show the developmental patterns seen in the raw frequency data, which confirms that changes in movement frequency are not driving developmental changes in synchrony profiles.

Second, despite changes in development, the profiles were found to be consistent across the 30-min observation period at each age (see Table 3 and Figure 6). Only at intervals typically not elevated above random (> 0.5 s) were small differences seen

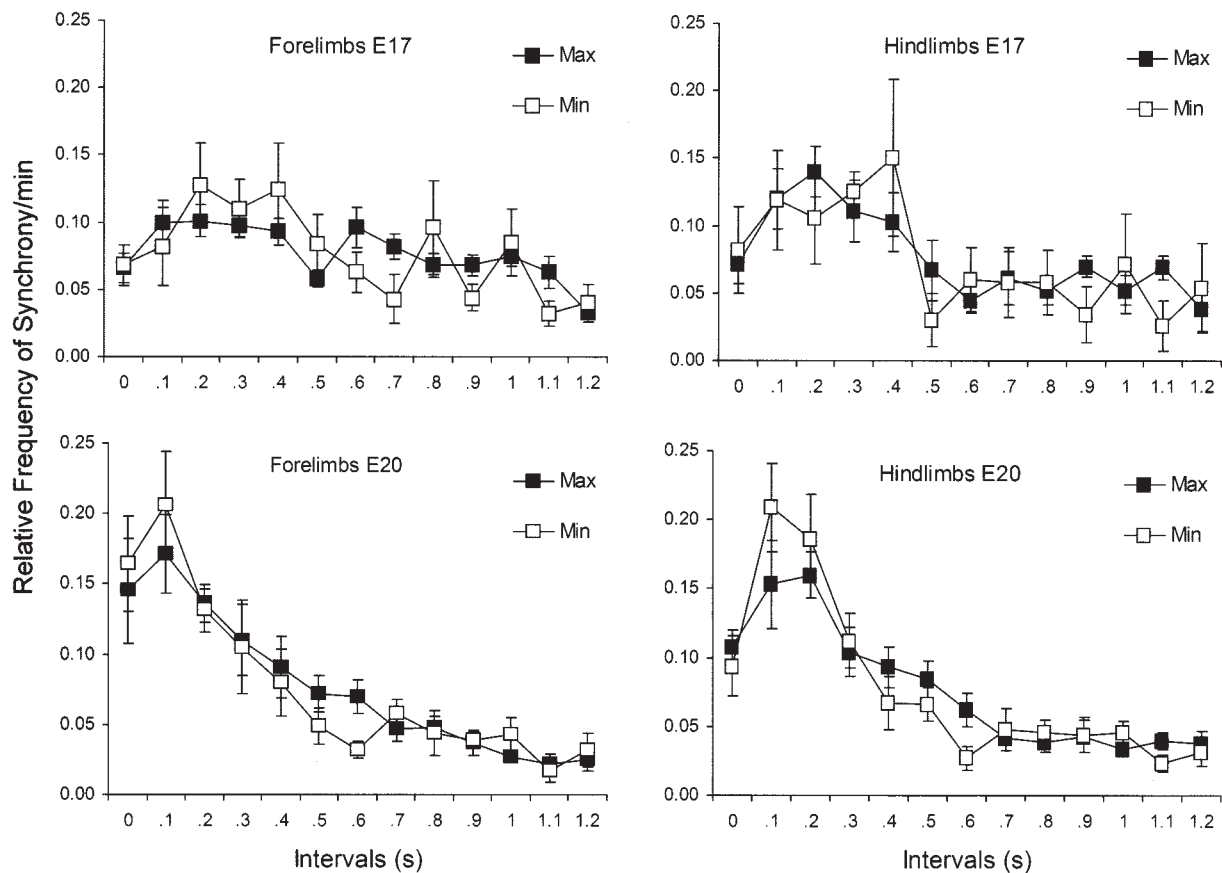


Figure 6. Profiles comparing the 5-min period of maximum and minimum interlimb movement synchrony for the forelimb and hindlimb pairs of fetuses at Gestational Day 17 (E17; top) and E20 (bottom). Points show the mean relative frequency of maximum (Max) or minimum (Min) synchronous movements per limb pair movement, expressed as rate per minute during observation, for each of the thirteen 0.1-s intervals. Error bars depict the standard error of the mean.

between profiles of minimum and maximum synchrony rates (see Figure 5).

Considering the robust nature of these movement patterns, profiles of synchrony may provide clues to the development and relationship between underlying neural circuitry and behavior. Earlier work with spontaneous movements, including their organization into clusters or bouts and modeling of the circuitry involved in this organization, revealed patterns of development similar to those seen with synchrony analysis (Robinson et al., 2000). The most striking similarity is that during fetal development, the probability of limbs moving in quick succession (defined by a criterion of 0.2 s or less) increased across gestation. Modeling of this developmental pattern suggested that descending circuitry plays a limited role, if any, in the organization of fetal movement bouts. Further, fetuses with high cervical transection of the spinal cord demonstrated the same bout patterns, despite lack of descending control from brainstem or cortical areas. Because of the similarities in the developmental changes in movement bouts and interlimb synchrony found in these studies, it is reasonable to assume that interlimb movement synchrony also is spinally mediated.

Although synchrony and bout organization may represent two ways of measuring the same (or closely related) process, the methods of quantifying interlimb synchrony in the present study reveal the coupling of specific combinations of limbs to a greater degree of precision than is possible in the bout analysis. Not only are changes in frequency seen across development, but changes in the distribution of those frequencies are seen as well. This detail allows a finer examination of developmental patterns. As would be expected by a typical rostral to caudal pattern of development, the forelimbs emerge with tight coupling earlier than any other limb pair combination. However, ipsilateral and contralateral synchrony does not emerge next, as might be expected in a purely rostral to caudal organization of the limb circuitry. Rather, the between-girdle combinations parallel developmental changes in the hindlimb pair. This simultaneous emergence suggests that the between-girdle ipsilateral and contralateral limb pair coordination may be tied more closely with hindlimb development than with a strict rostral-to-caudal gradient.

This finding may be because the ipsilateral and contralateral limb pair organization is controlled by communication between the forelimb and hindlimb girdles and not merely the result of adding additional limbs to the coordination of the forelimb pair. Hindlimb coupling, by this line of reasoning, needs to be in place for between-girdle communication to occur, which makes both forelimbs and hindlimbs centers of organization in the fetal rat. Such a dual control system has been suggested in a recent *in vitro* study of the developing spinal cord (Ballion, Morin, & Viala, 2001).

Further, development that is anchored by within-girdle coordination would be foundational for differential development of limb pairs, particularly the forelimbs. That forelimb synchrony coordination develops not only earlier but also in much greater isolation than hindlimb synchrony may reflect early differentiation, and perhaps functional specialization, of this limb pair. For example, numerous studies have demonstrated that by E20, when peak synchrony is first seen in the forelimbs, fetuses already possess a great degree of coordination in those limbs (Robinson & Smotherman, 1991, 1992b), as evidenced by the expression of action patterns such as facial wiping (similar to an adult grooming be-

havior), that is greater in many ways than they will develop at any later date in the hindlimbs. Such a differentiation of the forelimbs is not unusual and is seen in nearly all mammalian fetuses, but may be particularly evident in humans.

A parallel example of between-girdle differentiation is found in the studies of avian embryos, whose legs and wings will perform very different functions in adulthood. In younger chick embryos (E9), patterns of movement exhibit synchronies between the various limb combinations (wing to wing or wing to leg) that are about equal in frequency. As development progresses (E13), differential development between the wings and legs of the chick embryo takes place. Additionally, differentiation in the development of the movement patterns also occurs, and by a few days before hatching (E17), wing-wing synchronies predominate over wing-leg movements (Provine, 1980). This correlation between differentiation of limbs and coordination of movement between these limbs suggests that functional limb differentiation, as well as changes in limb allometry, may act as a selective pressure on experiential learning and the development of movement pattern.

However, the main question concerning the development of behavior and its underlying circuitry is not that differentiation occurs, but rather how it occurs. This question is ultimately subsumed by the experience versus maturation debate in the development of the nervous system. Because previous research has suggested that the observed synchronous movements are spinally mediated, it is tempting to propose that this coordination is simply a functional byproduct of the maturation of spinal circuitry. This hypothesis appears likely for several additional reasons. First, descending supraspinal projections to the spinal cord may be involved in the differentiation of synchronous patterns, although they may not be involved in their production. Descending fibers arrive continuously between E14 and birth (Lakke, 1997; Oudega, Lakke, Marani, & Thomeer, 1993) and could be temporally related to the changes seen in the development of synchronous coordination. Second, both the production of interneurons and intersegmental spinal projections also coincide with the acquisition of synchrony. However, as has been demonstrated through limb bud transplantation and synapse elimination experiments (Hamburger, 1975; Purves, 1988), activity plays an important role by maintaining and strengthening these neurons and their respective connections.

In addition to a rich description of spontaneous movement during development, measures of interlimb synchrony may be useful in several other modes of investigation. For example, because of its robust characteristics in typical development, synchrony measures may illuminate changes in neuromotor development brought about by perturbations to the developmental process itself. Typically, these perturbations would include maternal complications (e.g., diabetes, hypoxia, placental insufficiency, or oligohydramnios), and exposure to teratogens (e.g., irradiation, drugs of abuse, or environmental toxins). That investigation of nervous system development is traditionally confined to normal or typical development implies that only optimal developmental processes are being studied. In order to understand the full range of development that is possible, and hence gain a better understanding of the mechanisms involved in the process of development, atypical development must also be studied. Consequently, interlimb movement synchrony may prove useful in teratological studies for the advancement of both basic and applied research.

The ability to screen atypical movement patterns during fetal development, compared with typical developmental norms, may be useful in the early diagnosis of neurological disorders. Because there is a direct relationship between early diagnosis, treatment, and outcome in developmental disorders of the nervous system, such as cerebral palsy, the ability to detect nervous system abnormalities in the fetus is drawing increasing attention (Centers for Disease Control and Prevention, 1995). Current diagnostic techniques developed for human fetuses using qualitative measures of general movement (Einspieler, Prechtl, Ferrari, Cioni, & Bos, 1997) have had some success in assessing severe neurological dysfunction (Kainer, Prechtl, Engele, & Einspieler, 1997; Prechtl, 1997; Sival et al., 1997). These techniques rely on observer judgment to determine the quality of gross movements. The assessment and diagnosis of less severe forms of neurological dysfunction, however, may require a more exacting quantitative approach. Specifically, the analysis of fetal synchronous movement may provide the rich descriptive detail, as was found in the synchrony profiles of this study, that is needed for the precise assessment of neurological functioning.

Studies using fetal exposure to a known neurotoxin are currently underway to test the sensitivity of interlimb synchrony as an assessment measure (Kleven, Queral, & Robinson, 2001). Further, because these studies use a fetal rodent model, they may prove useful in identifying changes to specific patterns or a particular limb coupling that proves to be indicative of neural injury. Due to the restrictive visual and temporal resolution of current ultrasound techniques, being able to identify such an exemplar may be critical in applying the quantification of interlimb synchrony from the rodent model to human fetal ultrasound imaging.

This study suggests that the quantitative measure of interlimb movement synchrony is sensitive and robust and possesses a wide range of applicability for neurobehavioral research. Through these characteristics, this measure reveals clear developmental patterns of movement organization in the rat fetus. Further, interlimb movement synchrony reveals detail in the functional output of the developing nervous system that has been previously undetected.

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