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Spontaneous Motor Activity in Fetal and Infant Rats Is Organized Into Discrete Multilimb Bouts

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Spontaneous motor activity (SMA) is a ubiquitous feature of fetal and infant behavior. Although SMA appears random, successive limb movements often occur in bouts. Bout organization was evident at all ages in fetal (embryonic day [E] 17–21) and infant (postnatal day [P] 1–9) rats, with nearly all bouts comprising 1–4 movements of different limbs. A computational model of SMA, including spontaneous activity of spinal motor neurons, intrasegmental and intersegmental interactions, recurrent inhibition, and descending influences, produced bouts with the same structure as that observed in perinatal rats. Consistent with the model, bouts were not eliminated on E20 after cervical spinal transection, suggesting that the brain is not necessary to produce bout organization. These investigations provide a foundation for understanding the contributions of SMA to neuromuscular and motor development.

The earliest behavior of nearly all invertebrate and vertebrate animals is characterized by spontaneous, twitch-like movements of the head, trunk, and limbs (Corner, 1977; Hall & Oppenheim, 1987; Hamburger, 1963). Spontaneous motor activity (SMA), which is distinguished from reflexive movements in that it occurs without external stimulation, is a ubiquitous feature of behavioral expression and has been a primary focus of investigation of behavioral embryologists (Bekoff, 1976; Hamburger, 1973; Landmesser & O'Donovan, 1984; Narayanan, Fox, & Hamburger, 1971; Provine, 1973). SMA continues to be expressed after birth in the form of myoclonic twitches of the distal limbs that appear more rapid and jerky than the motor activity of fetuses; these infant twitches are phenomenologically similar to those occurring during REM sleep in adults (Blumberg & Lucas, 1996; Gramsbergen, Schwartze, & Prechtl, 1970). The possibility that these prenatal and postnatal movements may be causally and/or functionally related has been acknowledged for some time. For example, Corner (1977) stated that "sleep motility in its entirety . . . is nothing less than the continued postnatal expression of primordial nervous functional processes" (p. 292). Nonetheless, the hypothesis of developmental continuity between the SMA of fetuses and infants has rarely been directly addressed empirically.

The SMA of fetuses and infants exhibits nonrandom organization in both spatial and temporal dimensions. Circadian rhythms (De Vries, Visser, Mulder, & Prechtl, 1987), ultradian cyclicity (Robertson, 1987; Robertson & Bacher, 1995), and aperiodic clustering of activity (Robinson & Smotherman, 1988, 1992) are just a few examples of temporal patterns that are expressed across multiple time scales ranging from hours to minutes to fractions of seconds. One form of spatiotemporal organization that has received relatively little attention is movement synchrony, in which one limb moves in temporal proximity to another limb (Provine, 1980; Robinson & Smotherman, 1987). Although these synchronous movements occur predominantly at intervals of 0.5 s or less (Lane & Robinson, 1998), they are not simultaneous and do not resemble the whole-body startles that have long been recognized (Gramsbergen et al., 1970; Hamburger & Oppenheim, 1967). Furthermore, as we report in this study, movement synchrony reflects more than simply a temporal dependence among pairs of limbs; rather, patterns of movements among two or more limbs are organized into discrete bouts, where a *bout* is defined as the set of all limb movements in which the time interval between each successive limb movement does not exceed some criterion value (Fagen & Young, 1978; Machlis, 1977). When this bout analysis approach is used, similarities in bout structure between fetuses and infants become readily apparent, thus providing additional empirical support for Corner's (1977) developmental continuity hypothesis for SMA.

Despite the similarities in bout structure between fetuses and infants, the usefulness of the bout analysis approach will ultimately lie in its ability to provide insights into the underlying neural components that produce SMA, as well as its possible function. Therefore, in addition to quantifying the bout organization of SMA in fetal and infant rats, we describe a neural network model minimally configured to represent the spontaneously active elements within the spinal cord and brain that give rise to SMA. Specifically, the model represents spontaneous activation of spinal motoneurons, mutual interaction among motoneurons both within

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and between spinal segments, recurrent inhibition of limb activity, and descending influences from the brain. This model is sufficiently robust to capture the major features of bout organization as well as the developmental changes observed in rats from Day 17 of gestation to Day 9 postpartum. Moreover, one of the principal predictions from this model, namely, that descending influences from the brain play little or no role in generating multilimb bouts of motor activity, is tested in a final experiment in which rat fetuses are surgically prepared with a high cervical spinal transection.

Method

Subjects

Adult Sprague-Dawley laboratory rats (*Rattus norvegicus*) were maintained in a temperature-controlled room with a 12-hr light–dark cycle (lights on at 0600). Mothers were housed in standard laboratory cages (48 × 20 × 26 cm) in which food and water were available ad libitum. All subjects were treated in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, 1986).

In the initial study, 40 fetal subjects from 40 pregnancies were used. An additional 9 pregnancies provided 16 fetal subjects on Embryonic Day 20 (E20) of gestation to assess the effects of cervical spinal transection on fetal motor activity. Female rats were housed in groups of three with a single male during a 4-day breeding period. Vaginal smears were collected daily over the breeding period to determine whether conception had occurred; the first day in which sperm was detected was designated as E0 of gestation. With this breeding regimen, pregnant rats typically give birth on E22. Time-mated females remained housed together until fetuses were tested at one of five gestational ages (E17–E21).

Sixteen male and female infant rats from eight litters also were used. Female rats were housed in groups of two with two males during a 1-week breeding period, after which females were moved to individual cages for the remainder of gestation. The day of birth was designated as Postnatal Day 0 (P0), and, when necessary, litters were culled to 8 pups by P3. Finally, to control for the repeated handling and testing of pups in the longitudinal group, the motor behavior of an additional 8 pups from the same eight litters was recorded on P9 by using an identical procedure; these subjects are referred to as cross-sectional controls.

Preparation of Fetal Subjects

In the initial study, fetal subjects were tested at one of five gestational ages: E17, E18, E19, E20, or E21 (n = 8 at each age). All fetal subjects in the cervical transection study were tested on E20 (n = 8 per group). Detailed observation of fetal behavior was made possible by surgical procedures that produce spinal anesthesia in a pregnant rat and allow the uterus and fetuses to be externalized for direct visual access (Smotherman & Robinson, 1991). Under brief ether anesthesia, 100 μl of 100% ethyl alcohol was injected into the spinal canal between the first and second lumbar vertebrae. This procedure results in irreversible spinal anesthesia posterior to the site of injection. After spinal anesthesia, the pregnant rat was secured in a holding apparatus with a Velcro jacket, her uterus was externalized through a midline laparotomy, and the uterus and lower body were immersed in a temperature-regulated (37.5 ± 0.5° C) bath containing buffered physiological saline (Locke's solution). This benign fluid environment promotes fetal viability and allows detailed, direct observation of fetal behavior. The mother and fetuses were allowed to recover from the ether anesthesia in the water bath for 20 min before behavioral observations began.

After acclimation to the bath, an individual fetus was selected from each pregnancy for behavioral testing. The fetal subject was externalized from the uterus into the saline bath, and the embryonic membranes (chorion and amnion) were removed to permit experimental access to the fetus. At all times, the fetal subject remained connected by the umbilical cord to the placenta, which remained attached within the uterus. These procedures produce maternal and fetal preparations that are stable and resistant to deterioration; placental–uterine attachments are maintained in good condition for long periods, permitting observation sessions of 1–2 hr.

SMA of each fetal subject was videotaped continuously for either 15 min (E17 fetuses) or 30 min (E18–E21 fetuses). To gain clear visual access to limb movements, each fetal subject was positioned on its back with an elastic band across the thorax; this band was applied as loosely as possible while the fetus was held in a supine posture. Videotaping of fetal behavior was accomplished with a camera located above the water bath, and recordings were obtained in S-VHS format at the highest quality recording speed (SP; 60 fields/s), with simultaneous time code generation. The time code provided a visual time reference on each video field, permitting precise synchronization of frames during later playback and analysis.

Preparation of Infant Subjects

Eight infant subjects were tested longitudinally on P1, P3, P5, P7, and P9, with an additional 8 cross-sectional controls tested on P9. On the day of testing, the pup was removed from its home cage, weighed, and placed inside an incubator. All pups had fed recently, as evidenced by the presence of milk clearly visible through the abdominal skin. The incubator was maintained at an air temperature of 34–36° C and a relative humidity of 40–65%; the higher temperatures in this range were used with the younger subjects, the aim being to maintain pups within a thermoneutral environment (Spiers & Adair, 1986) that would be conducive to the expression of myoclonic twitching (Blumberg & Stolba, 1996; Sokoloff & Blumberg, 1998).

To gain clear visual access to limb movements, each infant subject was positioned on its back on a felt surface, and two elastic bands were secured around the pup. These bands were applied as loosely as possible while still maintaining the pup in a supine posture. In general, pups begin exhibiting myoclonic twitching of the distal limbs and tail within minutes of being secured inside the incubator.

A microcamera placed above the pup inside the incubator provided a clear view of all limb movements. Videotaping began 45–60 min after placement in the incubator and continued for 15 min. After recording was complete, the pup was marked for identification and returned to its home cage.

Procedures for Scoring Behavioral Data

Fetal and infant limb activity was scored from videotapes during normal-speed playback. To ensure high reliability in scoring movements, only one limb was scored in a given playback session; thus, four passes were made for each subject on each day to score the four individual limbs. Data were acquired with an event-recording program that records the identity and time of each keypress, with a resolution of at least 0.1 s. For each pass through a subject's video record, the videotape was cued to the same frame

and synchronized with the event recorder to permit subsequent reconstruction of a single time series comprising all limb movements.

For scoring motor activity in fetal subjects, each instance of movement, or unidirectional vector of continuous movement, was treated as a point event. Therefore, observers did not make distinctions among different kinds or magnitudes of limb movements. For scoring motor activity in infant subjects, two basic forms of movement were distinguished: *Twitches* were defined as phasic, rapid, and independent movements of a limb (Blumberg & Lucas, 1994; Gramsbergen et al., 1970); *awake* behaviors included coordinated and continuous motor activities such as stretching, kicking, and yawning.

Two experienced observers scored the fetal and infant motor activity. Each observer demonstrated a criterion level of proficiency before scoring data in this study. We have used similar scoring procedures in the past and have found them to be highly reliable, with inter- and intrarater reliability coefficients typically exceeding 0.85 (Blumberg & Lucas, 1994; Smotherman & Robinson, 1991).

Analysis of Fetal and Infant Motor Behavior

For analysis of fetal data, all movements were analyzed; for analysis of infant data, awake behaviors were edited out and only twitch movements were analyzed further. (Because awake behaviors in infant rats at these ages and under these conditions are relatively rare, their exclusion is not likely to have significantly influenced the results reported here. In all other respects, the fetal and infant data were analyzed identically.) Next, the four data sets obtained from scoring motor activity in each limb for each test session were interleaved to create a single continuous time series. This interleaved data set provided information about which limb had moved and the time of occurrence of each movement.

Analysis software written by one of the authors was used to analyze the bout organization of limb activity in each interleaved data set. In this report, a bout criterion interval of 0.2 s was used; this particular value was selected on the basis of preliminary analyses of these data. (It should be stressed that the basic finding of bout organization reported here is robust and is seen at bout criteria ranging from 0.1 to 0.5 s.) Finally, for each bout, the analysis program determined *bout length,* which was defined as the total number of limb movements in any particular bout.

A number of methods have been used previously for assessing and expressing bout organization (Fagen & Young, 1978; Machlis, 1977; Slater & Lester, 1982). For the present analysis, conditional probabilities were calculated for each fetal and infant subject to quantify the likelihood that, given the occurrence of y movements in an evolving bout, an additional limb movement would occur within the bout criterion interval to produce a bout of at least length $y + 1$. These *bout transition probabilities* were calculated by dividing the number of bouts of at least length $y + 1$ by the number of bouts of at least length y. Thus, separate estimates were calculated for the probability of adding a second movement to a bout (designated as "1..2"), adding a third movement ("2..3"), adding a fourth movement ("3..4"), or adding a fifth movement ("4..5"). The resulting series of transition probabilities are graphically depicted in "risk plots" and are computationally equivalent to age-specific survival or mortality data calculated from life tables used to summarize demographic data (Deevey, 1947). Because bouts comprising more than five limb movements rarely occurred, higher order transition probabilities are not included in the graphs presented below.

For fetal subjects, a repeated-measures analysis of variance (ANOVA), with event transition as the repeated measure and age as a between-groups factor, was used. For infant subjects tested longitudinally, a repeated-measures ANOVA, with both event transition and age as repeated measures, was used. When ANOVAs revealed overall significant effects at $p < .05$, post hoc paired or unpaired t tests were conducted to determine the source of these effects. Finally, differences between the P9 infants tested longitudinally and those tested cross-sectionally were tested with a repeated-measures ANOVA, with event transition as the repeated measure and testing condition (i.e., longitudinal vs. cross-sectional) as the between-groups factor. All means are presented with their standard errors.

Computational Modeling of Fetal and Infant SMA

To begin to understand the neural mechanisms that generate the observed temporal patterning of SMA, we developed a simple neural network model that generates a simulated stream of limb movements (see Figure 1). The model, written in Pascal, consisted of four virtual neurons that represented independent sources of neural activity within the spinal cord, each associated with one of the four limbs. A fifth virtual neuron represented the collective influence of neural activity descending from central sources in the brain. In addition to its role in inducing movement of its respective limb, each limb neuron was connected with each of the other three limbs by an excitatory synapse. Each limb neuron also produced an

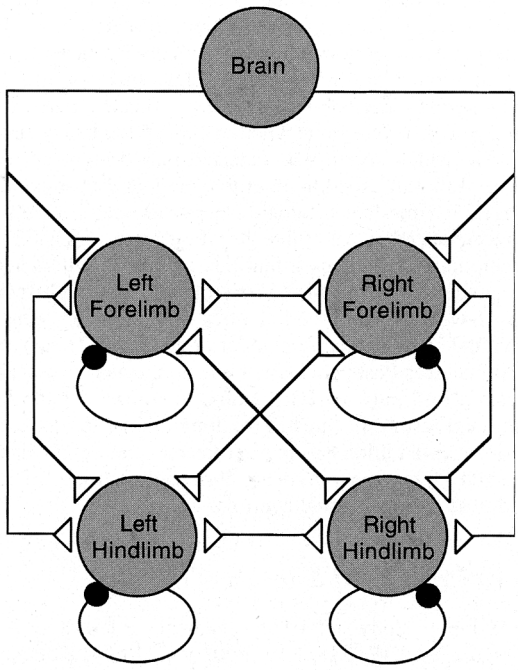

Figure 1. A "five-neuron" connectionist model incorporating a single motoneuron for each limb and a central neuron providing descending excitation to each of the four limbs. Each limb neuron exerts an excitatory influence on each of the remaining limb neurons via contralateral and ipsilateral connections. Moreover, each limb has a recurrent inhibitory connection. Spontaneous activity occurs in each of the five neurons according to a probabilistic equation. In total, there are 20 synaptic connections and five firing probabilities in this model. For nearly all of the present analyses, however, the five firing probabilities were set to the same value, and only 3 synaptic weights were varied: interlimb coupling (12 weights), recurrent inhibition (4 weights), and descending excitation (4 weights).

inhibitory effect on itself through a recurrent inhibitory synapse; the purpose of this recurrent inhibition was to create a functional refractory period for each limb neuron. Finally, descending projections from the central source were connected by excitatory synapses with each of the four limb neurons. Thus, the resulting network consisted of five spontaneously bursting neurons (four limbs and one central source) connected by 20 synapses (12 interlimb, 4 recurrent, and 4 from the central source). All synapses between limbs were considered to be excitatory, recurrent synapses were considered inhibitory, and synapses involving descending projections from the central source were considered excitatory.

The output of the model was computed in a continuous series of 0.1-s increments. That is, the net effect of intrinsic firing probabilities and postsynaptic potentials on the likelihood of an individual limb neuron firing was determined in each 0.1-s bin. The intrinsic firing probabilities of the four limb neurons and one central neuron were held constant in each bin. However, the strength of postsynaptic potentials was maximal in the interval immediately after the synaptic event (excitatory or inhibitory) and decayed exponentially thereafter. The model also permitted synaptic events at the same neuron to summate, thereby adjusting the probability of firing as a function of the recent history of synaptic events at that neuron.

A series of independent trials was completed to assess the general robustness and capability of the model to recreate features of bout organization expressed by fetal and infant rats. The intrinsic rate of spontaneous firing of limb neurons was adjusted to produce a time series of simulated behavior comprising approximately the same rate of limb activity normally expressed by fetuses and infants. To simplify assessment of a model with 25 parameters, the intrinsic firing rates of the five virtual neurons were not varied between trials. Although synaptic weights were systematically varied between trials, within any given trial all interlimb weights were set at the same value, all recurrent inhibitory weights were set at the same value, and all descending weights were set at the same value, yielding a model with three functional weight variables. Each trial yielded a simulated 30-min time series of limb activity; a sample of eight trials was conducted for every combination of synaptic weights examined. These simulated time series were analyzed and summarized according to the same procedures used to assess bout organization in the fetal and infant subjects. Despite its simplicity, systematic variation of the weights among the 20 synapses in the neural network yielded a surprising range of patterns of SMA, as described below.

Procedures for Preparing Fetal Subjects by Cervical Spinal Transection

To begin to understand how different regions of the developing central nervous system (CNS) contribute to the organization of spontaneous movements, a second experiment was conducted in which E20 fetuses were prepared by cervical spinal transection or a sham control procedure. Fetuses were prepared for behavioral observation as previously described. The spinal transection procedure was performed by inserting a microknife fashioned from a piece of fine stainless steel wire (0.15 mm diameter) into the cervical spinal cord at the base of the skull. The wire then was moved in a transverse plane to the left and right, completely severing the spinal cord between the foramen magnum and C2. Sham subjects were treated by inserting the wire adjacent to the spinal column without cutting across the cord. This method of transecting the CNS of the fetal rat is the same as that used in previous studies of fetal behavior (Robertson & Smotherman, 1990; Smotherman & Robinson, 1990). Sham and transected subjects were tested 30 min after the procedure in a 15-min experimental session, according to the same observation, scoring, and analysis procedures described above. At the conclusion of the testing session, subject fetuses were preserved in a 10% buffered formalin solution for later histological examination. The brain and spinal cord of each subject were sectioned in the midsagittal plane and examined under low magnification to determine the location and extent of the knife cut. All transected fetuses exhibited complete bilateral transection of the cervical spinal cord.

Results

Bout Analyses

For fetuses, rates of movement for the forelimbs and hindlimbs were 27.6 ± 0.9 and 18.3 ± 0.8 movements/minute, respectively. For the infants, twitch frequencies for the forelimbs and hindlimbs were 31.0 ± 0.9 and 23.9 ± 0.6 twitches/minute, respectively.

Figure 2 presents risk plots for the fetuses and infants. For the fetuses (left panel), a repeated-measures ANOVA indicated significant main effects of age, $F(4, 35) = 25.8$, $p < .0001$, and event transition, $F(3, 105) = 514.8$, $p < .0001$, as well as a significant Age × Event Transition interaction, $F(12, 105) = 9.9$, $p < .0001$. Post hoc tests revealed a number of interesting patterns. First, bout lengths greater than 4 were rare at all ages. Second, for bouts of length 4 or less, the probabilities of transition increased steadily with age. Third, for E18 and older fetuses, the probability of transition to a bout of length 3 was significantly greater than the probability of transition to a bout of length 2. And finally, for E20 and E21 fetuses, the probability of transition to a bout of length 4 was significantly less than the probability of transition to a bout of length 3.

Because the infant rats were tested longitudinally, it is important to establish that repeated testing did not influence any observed developmental trends. Indeed, there were no differences between the longitudinal and cross-sectional pups when they were compared at P9. Specifically, a repeated-measures ANOVA revealed neither a significant main effect of testing condition, $F(1, 14) = 2.4$, nor a significant interaction, $F(3, 42) = 0.1$. Therefore, only the longitudinally tested pups are discussed further.

For the infant rats, a repeated-measures ANOVA indicated significant main effects of age, $F(4, 84) = 7.0$, $p < .001$, and event transition, $F(3, 84) = 243.7$, $p < .001$, as well as a significant Age × Event Transition interaction, $F(12, 84) = 6.1$, $p < .001$. As with the fetuses, post hoc tests revealed a number of interesting patterns, some of them involving trends opposite to those found in the fetuses. First, bout lengths greater than 4 were again rare at all ages. Second, for bouts of length 4 or less, probabilities of transition decreased steadily with age. Third, only for P1 infants (the youngest postnatal age tested) was the probability of transition to a bout of length 3 significantly greater than the probability of transition to a bout of length 2. Finally, at all postnatal ages, the probability of transition to a bout of length 4 was significantly less than the probability of transition to a bout of length 3.

The low probability of bouts with lengths greater than 4

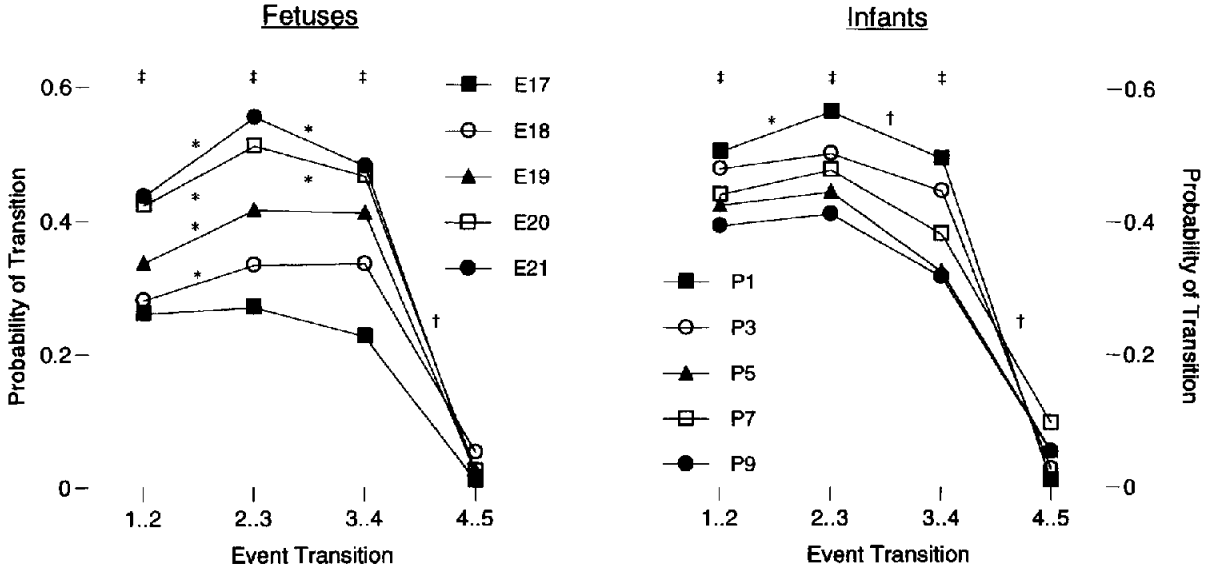

Figure 2. Risk plots. Each plot depicts the probability of adding an additional limb movement to a bout, which is defined as a group of limb movements in which each successive movement within the group occurs no more than 0.2 s after the previous movement. Data are means from rat fetuses and infants at each of five different ages. †Significant difference between adjacent points at all ages; *significant difference between the indicated adjacent points only; ‡significant difference between the oldest and youngest (i.e., Embryonic Day [E]17 vs. E21, Postnatal Day [P]1 vs. P9) rats at the indicated event transition. Despite the use of different methodologies and different behavioral scoring procedures, clear developmental continuities are evident across the birth transition. Standard error bars are included in the figure but are smaller than the symbols.

suggests that each of the 4 limbs contributed once and only once to each bout. Closer inspection of the data revealed that this was indeed the case. Across all ages, the mean percentage of bouts in which the same limb moved more than once was 0.5% (range: 0.025–1.4%).

Computational Model

As shown in Figure 1, the model used here to simulate SMA is composed of five neurons and 20 synaptic weights. To simplify the present analyses, however, the five intrinsic firing probabilities were set to the same value and only three functional synaptic weights were varied: interlimb coupling (12 weights), recurrent inhibition (4 weights), and descending excitation (4 weights).

Figure 3 summarizes data from a series of trials generated by this model that were analyzed by the same methods applied to fetal and infant subjects (Figure 2). Each of the six panels in the figure presents data for three levels of interlimb coupling (weak, moderate, and strong), organized in a 3 column × 2 row matrix. The three columns represent different levels of descending excitation from the central source (none, moderate, and strong excitation), and each row represents either weak or strong recurrent inhibition. Every point depicts the mean of eight independent trials of the model with a particular set of values for these three variables.

When compared with the data in Figure 2, a number of features are apparent. First, strong recurrent inhibition is necessary to prevent the same limb from contributing more than once to a bout, and thus is responsible for producing the sharp decline in transition probability for the 4..5 event transition. Second, increases in the strength of limb coupling (interlimb synaptic weights) resulted in a systematic increase in transition probabilities in all 6 plots, suggesting that changes in coupling strength may account in part for age-dependent changes observed in the fetal and infant rats. Third, increases in descending excitation also produced an increase in transition probabilities, especially at the weak and moderate limb coupling values. Finally, probabilities generally increased from the first to the second event transition (i.e., 1..2 to 2..3) and decreased from the second to the third event transition (i.e., 2..3 to 3..4) across a range of combinations of limb coupling and descending excitation.

To examine the possibility that descending excitation and recurrent inhibition alone might account for the fetal and infant data presented in Figure 2, an additional simulation was performed in which the intrinsic firing probabilities and interlimb coupling weights for the four limb neurons were set to 0. The results of this simulation are presented in Figure 4 for weak, moderate, and strong descending excitation of the four limb neurons. In this simulation, bout structure was evident only when descending excitation was great enough to produce transition probabilities approaching 1; even though strong recurrent inhibition remained an element of this simulation, transition probabilities did not show an abrupt decrease at event transition 4..5 with weaker levels of descending excitation. In addition, with the present model, it was not possible to generate probabilities at event transitions

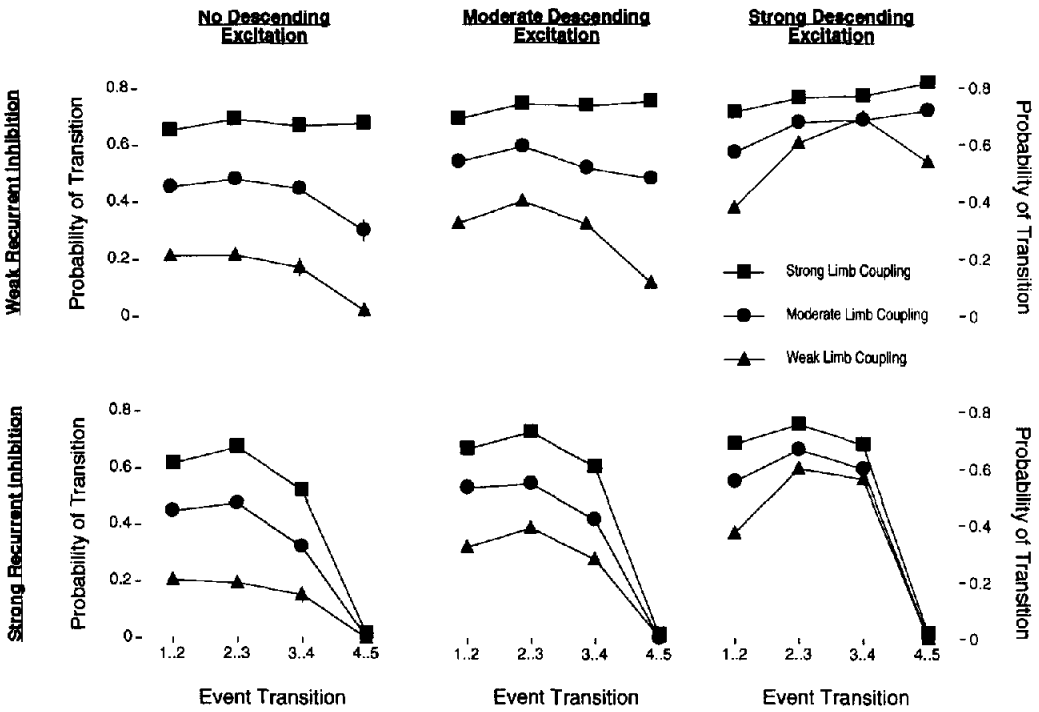

Figure 3. Risk plot simulations using the connectionist model described in Figure 1. For this figure, synaptic weights were varied across three dimensions: First, each of the six plots presents three levels of interlimb excitatory coupling, weak (triangles), moderate (circles), and strong (squares); second, each column presents a different level of descending excitation (none, moderate, and strong); and finally, each row presents a different level of recurrent inhibition (weak and strong). Each point represents the mean value of eight independent trials. When compared with the data in Figure 1, it is apparent that bout structure depends on the presence of strong recurrent inhibition. Moreover, other changes in the structure of the risk plots (e.g., increases and decreases in probability transitions) may result from a combination of changes in interlimb coupling and descending excitation. Standard error bars are included in the figure but are smaller than the symbols.

of 2..3 or 3..4 that were greater than the first event transition (i.e., 1..2). Finally, at the lower levels of transition probability that are characteristically expressed by fetuses and infants, bout structure was absent and the risk plots declined monotonically.

Effect of Cervical Transection on Bout Structure in E20 Fetuses

To test the model's prediction that descending influences from the brain are not necessary to produce multilimb bouts of SMA, the behavior of E20 fetuses after high cervical transection was analyzed. Histological examination of experimental subjects confirmed that all had complete transection of the spinal cord between the base of the brain and C2. Figure 5 is a photograph of a cross-section of the head region of a representative E20 fetus with a high cervical transection. The arrow indicates the level (i.e., C1) and extent of the transection.

High cervical transection did not significantly influence the number of forelimb or hindlimb movements exhibited by the E20 fetuses. Specifically, across the 15-min observation periods, sham-transected fetuses produced 26.0 ± 2.8 forelimb movements/minute, and transected fetuses produced 25.4 ± 3.7 forelimb movements/minute. The respective values for hindlimb movements were 20.1 ± 3.0 and 14.9 ± 3.4 movements/minute.

Figure 6 presents the risk plots for the two experimental groups. It is apparent that high cervical transection did not prevent limb activity from clustering together into multilimb bouts. A repeated-measures ANOVA indicated a significant main effect of event transition, $F(3, 42) = 26.3$, $p < .0001$, but not of group, $F(1, 14) = 0.2$, and the Event Transition × Group interaction also was not significant, $F(3, 42) = 1.1$. Because these last two tests were not significant, the data were collapsed before post hoc tests were performed. As indicated in the figure, event transitions decreased significantly at the 4..5 transition, characteristic of the bouts presented in Figure 2. Finally, to determine whether there was a significant decrease at the 4..5 transition for the transected fetuses alone, a single one-tailed planned comparison was performed; this test indicated a significant reduction in the 4..5 transition probability, $t(7) = 2.1$, $p < .05$, providing strong evidence of bout structure for these fetuses.

The finding here that high cervical transection did not alter the bout structure of SMA does not mean that all aspects of behavior were unaffected by the loss of descend-

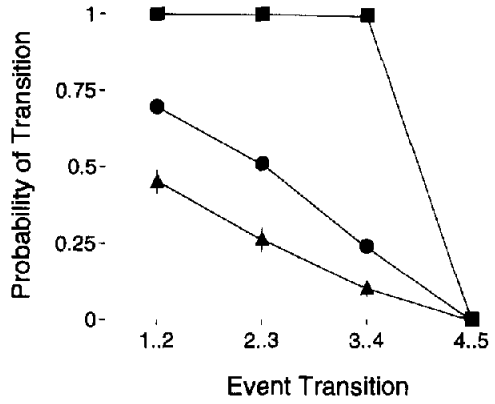

Figure 4. Risk plot simulation for the connectionist model set up for descending excitation and recurrent inhibition only. For this simulation, firing probabilities for the four limb neurons and all limb coupling weights were set to 0. Recurrent inhibition was set at the highest level shown in Figure 3. The three graphs represent three synaptic weights for descending excitation to the four limbs, from weak (triangles) to moderate (squares) to strong (squares). Each point represents the mean value of eight independent trials. It is clear that descending excitation alone is not capable of generating the bout structures evident in Figure 2. Standard error bars are included in the figure but are smaller than the symbols.

ing influences from the brain. Indeed, there appeared to be changes in the quality of the movements, including reduced movement amplitude, in transected subjects. Such features, however, were not explicitly measured in this experiment.

Discussion

The present results indicate the presence of previously undetected structure in the SMA of fetal and infant rats. As shown in Figure 2, the probability of an additional limb movement occurring within the criterion interval of an existing bout remains relatively constant until all four limbs have moved, then sharply declines. Bout organization, as we refer to this form of temporal patterning of SMA, appears to

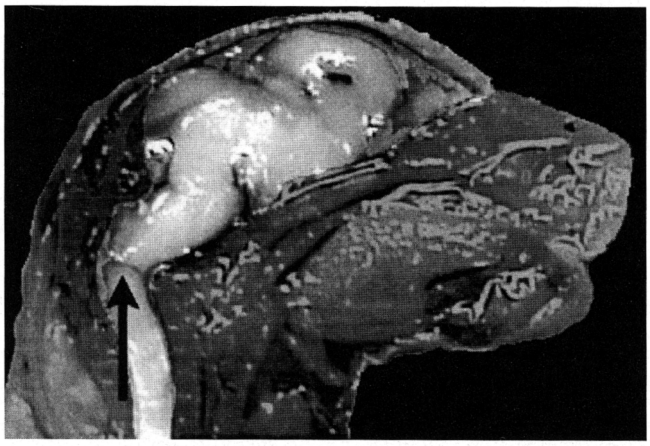

Figure 5. Cross-section of a representative 20-day-old fetus with a high cervical transection. The arrow indicates the level of the transection.

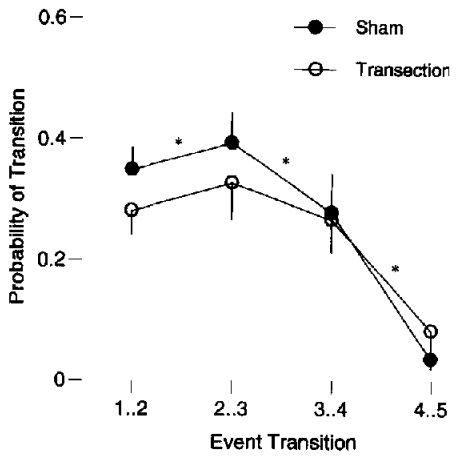

Figure 6. Risk plots for 20-day-old fetuses that experienced high cervical (open circles) or sham (filled circles) transections. *significant difference between adjacent points for collapsed data. Values are means (±*SEM*).

be a highly robust phenomenon that is consistently expressed by fetal and infant rats, and it exhibits systematic changes during prenatal and postnatal development (see Figure 2).

According to our scoring procedures, the same limb was very unlikely to move more than once within a bout. This could suggest that the limb acts as a single unit regardless of the character of the movement (i.e., whether the movement involves joint excursion at the shoulder, elbow, wrist, or digits). However, more detailed analytic approaches, such as frame-by-frame kinematic analysis or electromyographic recording from multiple muscle groups within a limb, will be required to evaluate this possibility.

It is not known what neural elements are responsible for generating the distinctive temporal pattern of activity that is evident in interlimb bout organization. It long has been known that isolated segments of the cervical and lumbosacral spinal cord are capable of generating SMA (Hamburger, Wenger, & Oppenheim, 1966). In the avian embryo, for instance, limb movements are associated with polyneuronal burst discharges that are widely distributed within the spinal cord (Provine, 1971; Ripley & Provine, 1972). These bursts of neuronal activity can occur nearly simultaneously across many spinal segments, especially at the beginning of an episode of spontaneous motor activity; brief, high-amplitude potentials recorded from ventral roots have been referred to as the "initiating discharge" (Provine, 1972) or "synchronous discharge" (Landmesser & O'Donovan, 1984). Similar bursts of intersegmental activity have been reported in fetal sheep early in gestation (E46) but are not evident in older fetuses (E96; Berger, Kyriakides, & Cooke, 1997).

A spinal source for SMA is further suggested by findings that cyclic and noncyclic temporal patterning is not abolished by caudal to midthoracic spinal cord transection in chick embryos (Bradley & Bekoff, 1992; Ho & O'Donovan, 1993), fetal sheep (Berger et al., 1997), and fetal and infant rats (Blumberg & Lucas, 1994; Robertson & Smotherman, 1990). In the studies on fetal and infant rats, midthoracic

transection reduced hindlimb twitching by 40–50%, suggesting that descending influences are responsible for approximately half of all SMA below the level of the transection. It could not be determined from these studies, however, whether these descending influences originate in the spinal cord or the brain. The present finding that high cervical transection in E20 fetuses neither reduces limb activity nor abolishes bout organization of SMA provides direct evidence that, at least in fetuses, the spinal cord alone is sufficient to organize many aspects of SMA.

The computational model described here and illustrated in Figure 1 may help to guide our understanding of the mechanisms underlying SMA and its bout structure. Specifically, the simulations presented in Figure 3 suggest a number of possibilities: First, the bout structure evident in the SMA of fetal and infant subjects may be dependent on some form of recurrent inhibitory feedback. Second, much of the complexity that we have observed in the temporal patterning of SMA can be mimicked without including descending influences from the brain, as confirmed by the data from E20 fetuses with high cervical transections. Indeed, in the model, descending excitatory activity alone, or presumably any single source of activity, is inadequate to produce limb activity that is organized into bouts (Figure 4).

The age-related changes in bout structure presented in Figure 2, when compared with the modeled data in Figure 3, suggest some developmental hypotheses. For example, transition probabilities increased systematically between E17 and E21 and then decreased from P1 to P9. If, as appears to be the case in fetuses, descending excitation from the brain is not playing a role in bout organization, then the model suggests that interlimb coupling increases across fetal development, perhaps reflecting changes in collateral motor efferents (i.e., efference copy) or propriospinal feedback (i.e., reafference). Similarly, postnatal decreases in transition probabilities could be due to a reduction in interlimb coupling.

In a recent study, the patterning and neurochemical bases of spontaneous activity by ganglion cells in the embryonic chick retina was investigated (Wong, Sanes, & Wong, 1998). Using an in vitro preparation, Wong and colleagues used optical and electrophysiological techniques to examine the waves of spontaneous activity that are thought to play a role in neuronal development and topographic organization. In their observations of this spontaneous activity, the authors note that "bursting activity renders [ganglion cell layer] cells refractory to subsequent activation for a considerable period" and that "cells never burst twice in rapid succession as would result if a wave turned back to propagate through its own wake" (p. 8843). This observation of a ganglion cell refractory period may be analogous to our observations that spontaneous limb movements occur in bouts, each of which is nearly always composed of the activity of different limbs, implying a functional refractory period after each limb movement. In our model, this refractory period is instantiated as a recurrent inhibitory loop, but the neural circuitry underlying this recurrent inhibition is unspecified. Possible mechanisms include Renshaw cell modulation within the spinal cord (Ashby, 1995) or a functional refractory period of individual motoneurons or electrotonically coupled networks of motoneurons (Kandler & Katz, 1995). Regardless of the exact mechanism, however, refractory periods may be a general feature of spontaneously firing neuronal circuits that is expressed at both the cellular and behavioral levels.

Spontaneous neural activity is now recognized as a vital source of information to the developing nervous system (Colman & Lichtman, 1993; Shatz, 1990). For example, spontaneous firing of retinal ganglion cells in rats in utero, more than 2 weeks before eye opening occurs, plays a central role in the neural organization of the visual system, including the development of ocular dominance columns and topographic organization (Galli & Maffei, 1988). On the basis of recent computational models of visual system development, it appears that spontaneous activity is a driving force behind unsupervised neural organization in higher order structures such as the lateral geniculate nucleus and area V1 of the occipital cortex (Erwin & Miller, 1998; Miller, 1990; Miller, Keller, & Stryker, 1989). The establishment of orderly connections in the developing motor system entails many of the same kinds of problems faced by developing sensory systems, suggesting that similar developmental mechanisms may be involved. Thus, spontaneous activity of motoneurons in the spinal cord and brain may provide information, analogous to the spontaneous activity of retinal ganglion cells, that helps to guide the development of neuromuscular connections and topographic organization, as well as the maintenance of this organization in adults (Blumberg & Lucas, 1996).

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