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Environmental determinants of behaviour in the rat fetus. II. The emergence of synchronous movement

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Abstract. Rat fetuses were observed directly in different micro-environments (in utero and ex utero) on successive days during the last third of gestation. Synchronous movements comprising two or more regions of the body became common on day 17 of gestation and were especially prevalent ex utero. Their occurrence could not be explained satisfactorily by a stochastic model involving chance association of individual body regions. If synchronized movements are considered as evidence of behavioural organization, then fetal behaviour appears more diverse than previously recognized and exhibits specific linkages between body regions that imply emergent coordination and the prenatal development of species-typical action patterns.

Information obtained with the aid of recent improvements in technology and surgical technique is promoting a new view of the fetus as behaviourally competent. In our laboratory, we have found it possible to observe directly the behaviour of rat fetuses, *Rattus norvegicus*, immersed in a fluid medium (Smotherman et al. 1986a). Observation of rat fetuses under various environmental conditions has been employed successfully to study the normal development of spontaneous fetal behaviour (Narayanan et al. 1971; Smotherman et al. 1984), the influence of the intra-uterine environment on fetal activity (Smotherman & Robinson, in press), the ability of fetuses to learn (Smotherman & Robinson 1985), the emergence of sensory responsiveness during the prenatal period (Smotherman & Robinson 1987b), and the effect of teratogenic substances, such as alcohol, on fetal activity (Smotherman et al. 1986b).

Understanding some of the variables that influence spontaneous fetal behaviour has been facilitated by observation of rat fetuses in one of three physical conditions: within the uterus, externalized from the uterus while remaining within the amniotic sac (with placental attachment unimpaired), and externalized from both the uterus and the amnion. It is feasible to observe fetuses during the last third of gestation in all three micro-environments (Smotherman & Robinson 1986). The first subtle movements of limbs begin on day 16 of gestation (with day 0 defined as the detection of sperm in a vaginal smear). Thereafter, fetal behaviour undergoes progressive levels of organization, culminating just before parturition in diverse head, limb and trunk movements that exhibit temporal clustering, synchrony and short-period rhythmicity (Robertson, Smotherman & Robinson, unpublished data). The degree to which behavioural organization is evident and the earliest age of its emergence are strongly influenced by the conditions of observation. Fetuses under the greatest physical restraint (In Uterus) exhibit less patterning than unrestrained fetuses (In Bath).

One aspect of emerging behavioural organization in the fetal rat is movement synchrony. In this paper, the movement of two or more body parts is considered synchronous if the onset of their movement is simultaneous. On days 16 and 17 of gestation, synchronous movements are not more frequent than expected by chance association of different body parts. By day 18, however, synchronous movements become more abundant than can be accounted for by chance. Synchronous movements also become relatively less common under conditions of physical restraint in utero. The effect of restraint is more evident when fetuses are observed within the uterus on day 21, when preterm body size is maximal and amniotic fluid volume is minimal, particularly if many fetuses reside in the same uterine horn (Smotherman & Robinson 1986).

In these previous approaches to characterizing fetal behaviour all events involving synchronous movement were considered equivalent and collapsed into a single category for analysis (Smotherman & Robinson 1986). In the present study, we extend the analysis to discriminate between different kinds of synchronous movement. First we present evidence that certain synchronous movements occur more frequently than expected, implying that some patterns of fetal behaviour are not accidental combinations of individual body parts. Then we proceed to describe how different body parts are incorporated into synchronous movements and how developmental age and fetal microenvironment influence evident linkages between body parts and the overall repertoire of fetal behaviour. We view these patterns of movement synchrony as an important aspect of the epigenesis of behaviour during the prenatal period which ultimately leads to coordinated movement and the emergence of discrete action patterns.

GENERAL METHODS

Subjects

Subject fetuses were the progeny of female Sprague–Dawley rats bred to Long–Evans males (Simonsen Laboratories. Gilroy, California). Females were housed in polycarbonate cages ($32·7 \times 37·8 \times 9·5$ cm) under a 12:12-h L:D cycle in compliance with DHHS (NIH) (1985) and ASAB–ABS (1986) guidelines for animal care.

Preparation of Mother and Fetuses

Pregnant females were prepared surgically to eliminate sensation from the lower body while circumventing the suppressive effects of general anaesthesia on fetal behaviour. Under ether anaesthesia, each female received an injection of 2% lidocaine with epinephrine (0·001%) into the spinal cord between the first and second lumbar vertebrae. The resultant spinal anaesthesia was effective in immobilizing the hindquarters of the female for 60–90 min, providing ample time for observation of fetuses. This method of reversible anaesthesia is not detrimental to the mother or fetuses, as evidenced by the ability of prepared females to carry litters to term and produce healthy offspring (Smotherman et al. 1986a). Following the spinal injection, each female was restrained in a Plexiglas holding apparatus (Smotherman et al. 1984), her uterus was exteriorized through a midline laparotomy, and her uterus and hindquarters were immersed in a temperature-controlled (37·5° ± 0·5°C) bath containing isotonic saline (Locke's solution, Galigher & Kozloff 1971).

The mother and fetuses were allowed to recover from ether anaesthesia and acclimate to the water bath for 20 min before behavioural observation began. Two healthy fetuses were selected as subjects from each mother and were prepared in one of three ways for direct observation of behaviour: within the uterus, within the amniotic sac, or externalized into the saline bath (Smotherman & Robinson 1986). Each fetus was observed in one of the three fetal preparations (In Uterus, In Amnion or In Bath) at one of six gestational ages (days 16–21). In each cell of the 3-preparation × 6-age factorial design, six pregnant females provided two subject fetuses. Because fetuses on day 16 exhibited synchronous movements very rarely, only fetuses 17–21 days of age provided data for model testing and general description of behavioural synchrony ($N = 180$ fetuses). For measurement of changes in behavioural diversity and repertoire, however, day 16 fetuses were included in the analysis ($N = 216$ fetuses).

Data reported in this study are exclusively from fetuses that remained healthy throughout the observations (Smotherman & Robinson 1986). While the maternal preparation is reversible and permits completion of gestation (Smotherman et al. 1986a), in this study adult rats were humanely killed subsequent to observation, obviating post-surgical recovery, and fetuses were delivered by Caesarean section and variously used in studies of morphological development (Smotherman & Robinson, in press) and preterm/postnatal behaviour (Smotherman et al. 1987).

Behavioural Observation

The behaviour of the two subject fetuses was observed during consecutive 10-min periods. Fetal behaviour was recorded by an observer, who watched the subject fetus and categorized fetal behaviour, and a scribe, who entered the data into a real-time event recorder and monitored bath temperature. After observation, the number of behavioural events in each category was tallied; frequency counts were the basis for quantitative analysis. This protocol has been found to be reliable in preserving a continuous sequential record of fetal movements (Smotherman & Robinson 1985).

Categories of Behaviour

Observed fetal activity was categorized with reference to the region of the fetus' body that moved. Fetal movements were described in terms of seven body regions: foreleg (F), rearleg (R), head (H), mouth (M), curl (C; a ventral or lateral flexion or torsion of the body trunk), stretch (S; a dorsal extension or straightening of the trunk) and twitch (T; a spasm along the side of the trunk in the vicinity of the diaphragm). See Smotherman & Robinson (1986) for a discussion of the age of first occurrence and subsequent patterns of development of these body regions.

Body regions could be moved singly or in any combination. Bilateral and unilateral movements of a particular body region (e.g. forelegs) were not distinguished. This allows for 120 possible categories of synchronous movement resulting from combination of the seven body regions (2^7 combinations $(=128)-7$ single -1 no movement $=120$ synchronous). As a convention, each category of synchronous movement is uniquely represented by the first letter of its constituent body regions (e.g. C consists of a trunk curl alone, and CFR consists of synchronous curl, foreleg and rearleg movement). Because synchronous movements were defined by having simultaneous onset, no order or sequential relationship is implied by the series of letters in the descriptive label (i.e. CFR is equivalent to FRC).

Of the 120 categories of synchronous movement, the eight most frequent were distinguished for the purpose of testing a random model of behavioural synchrony. These eight (CF, FH, FHM, FHR, FM, FR, HM and HR) constituted 94% of all observed synchronous movements. The remaining 6% of synchronous movements were distributed among 26 categories, which due to their infrequent occurrence were collapsed into a single grouping, OTHER, for model testing.

Random Association Model of Behavioural Synchrony

The random model of behavioural synchrony presented in our earlier paper (Smotherman & Robinson 1986) attempted to describe the occurrence of synchronous movements as the chance association of body regions. In the first approach, the expected probability of a synchronous movement was calculated as the joint probability of movement of two or more body regions. Because the probability of a regional movement (of any type) was estimated from overall fetal activity (the total number of regional movements divided by the number of 1-s intervals in which movement could occur), the model assumed a relationship between the frequency of synchronous movement and the rate of fetal activity. In contrast, the probabilistic model presented in this paper makes no such assumption that synchronous movements are the result of accidental temporal conjunction of body regions. That is, it does not consider the amount of time devoted to observation or the rate of fetal activity. Rather, it assumes that the frequency of occurrence of a particular synchronous category is proportional to the overall frequency with which the constituent body regions are moved. This model makes more specific predictions and generates contingent probabilities (i.e. given the occurrence of 100 synchronous movements, what is the predicted frequency of category x?).

If $M(\mathrm{r})$ is the number of movements involving body region r, n is the number of body regions ($n=7$), and $i=(1, 2, 3, \ldots, n)$, then the long-run probability that a given synchronous movement includes body region r may be estimated from the equation

$$P(\mathrm{r}) = \frac{M(\mathrm{r})}{\sum_{i=1}^{n} M(i)} \quad (1)$$

Similarly, the probability that a given movement does not involve body region r is the complement of $P(\mathrm{r})$: $P(\bar{\mathrm{r}}) = 1 - P(\mathrm{r})$.

With these probabilities calculated for all seven body regions for each fetus, a product model can be used to generate probabilities of various combinations of body regions. Thus, the probability of a movement including regions r_1 and r_2, and only these two regions, can be calculated as the product of the probabilities of movement of these two regions times the product of the complements of all other regions

$$P(\mathrm{r}_1\mathrm{r}_2) = P(\mathrm{r}_1) \times P(\mathrm{r}_2) \times P(\bar{\mathrm{r}}_3) \times P(\bar{\mathrm{r}}_4) \ldots \times P(\bar{\mathrm{r}}_n) \quad (2)$$

The expected frequency of a given category of synchronous movement may then be computed for each individual fetus as the product of the synchronous probability $P(\mathrm{r}_1\mathrm{r}_2)$ and the observed number of synchronous movements for that particular fetus.

Because expected frequencies of nine focal categories of synchronous movement were calculated for each of the 180 subjects (3 preparations × 5 ages × 12 fetuses), the behaviour of each individual fetus provided an independent case for testing the model. Within each age × preparation group, for each of the nine focal categories, a chi-squared test was used to assess significant deviations from frequencies of synchronous movements predicted by the model.

Descriptive Analysis of Synchronous Movement

The number of different categories of movement exhibited by each fetus provided an index of overall repertoire size. Behavioural diversity, reflecting both the number of different categories and the frequency of each, was quantified using a standard information theory measure of entropy (Shannon & Weaver 1949)

$$H = \sum_{i=1}^{n} P_i(-\log_2 P_i) \qquad (3)$$

where n is the number of observed categories, $i = (1, 2, 3, \ldots, n)$, P_i is the probability that a given movement will consist of category i, and H is the overall entropy or diversity of behaviour expressed in bits per movement. H varies from a value of 0 (when only one behavioural category occurs) to a maximum of $\log_2 n$ (when all categories are equally probable). Movements involving both single and multiple body regions are included in analyses of repertoire size and behavioural diversity, in contrast to our previous paper which considered only the seven basic body regions.

Two additional analyses were employed to characterize further the relationships among specific regional movements. These indices were computed separately for fetuses observed on days 17–21 of gestation in each of the three fetal preparations. An index of affinity was calculated to express the likelihood that a particular body region would become involved in a synchronous movement. Affinity was defined as the number of synchronous movements involving body region x divided by the total number of movements (single and synchronous) involving region x. A more detailed index of linkage was used to reflect patterns of association between specific body regions. Linkage was computed as the total number of times two given regional movements occurred in synchrony (regardless of other associated regions).

Where appropriate, data were analysed by two-factor between-subjects analysis of variance. ANOVAs were followed by tests for simple main effects and post-hoc tests using the method of Neuman–Keuls (Winer 1971). Becauses analyses of variance and chi-squared tests were performed on multiple dependent variables, a conservative alpha level (0·01) was considered significant in all tests.

RESULTS

The raw data in this paper were the subject of the first paper in this series (Smotherman & Robinson 1986). In our original treatment of these data, a series of analyses found no evidence that infrequent movements by the restrained mother or the sex of the subject fetus influenced fetal activity or several measures of behavioural organization. Moreover, an analysis which took into account a litter factor (two fetuses from the same mother were tested) failed to indicate this factor as a significant source of variation. For this reason, the two fetuses observed in each litter were considered independent in the following behavioural analyses.

Rat fetuses begin to perform spontaneous movements on day 16 of gestation; the level of fetal activity is influenced by gestational age (rising to a peak on days 18–19) and environmental condition at the time of observation (fetuses In Bath generally are more active than In Uterus). Smotherman & Robinson (1986) provide a more complete discussion of these patterns of developmental change and environmental influence on overall fetal activity.

Synchronous movements by fetal rats were uncommon on day 16 of gestation; a total of 32 synchronous movements was observed among 36 subjects at this age ($\bar{X} = 0{\cdot}89$ movements/fetus/10 min observation). In contrast, 180 fetuses 17–21 days of age exhibited a total of 4890 synchronous movements ($\bar{X} = 27{\cdot}2$ movements/fetus/10 min). Because synchronous movements did not become common until day 17, our analysis and description of behavioural synchrony is restricted to these older fetuses.

Tests of the Random Association Model of Synchrony

For the first 2 days following the onset of motility (16–17), the incidence of synchronous movements can be predicted from the amount of

Table I. Deviations from a random association model of synchronous fetal activity

Age	Preparation	CF	FH	FM	FR	HM	HR	FHM	FHR	OTHER
		Category of synchronous movement*								
17	In Uterus		+							
	In Amnion				+	+				
	In Bath				+	+	+			–
18	In Uterus					+				
	In Amnion					+		+	–	–
	In Bath				+	+	–	+		–
19	In Uterus									
	In Amnion		+		+					–
	In Bath				+	+			+	–
20	In Uterus		+							
	In Amnion		+		+					–
	In Bath		+		+		–			–
21	In Uterus									
	In Amnion				+	+				–
	In Bath				+	+	–			–

Entries in the table indicate a significant deviation from predicted frequencies in a chi-squared analysis ($P < 0.01$). The direction of deviation is indicated by the symbol (+ = more frequent than expected; – = less frequent).
* See text for definition of categories.

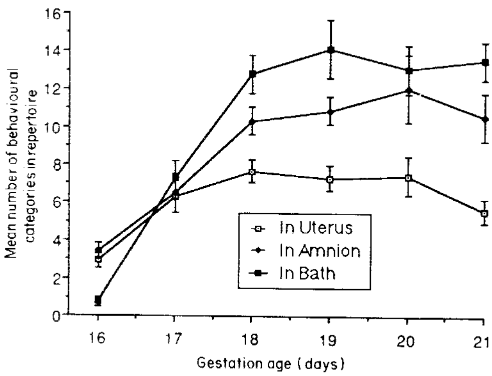
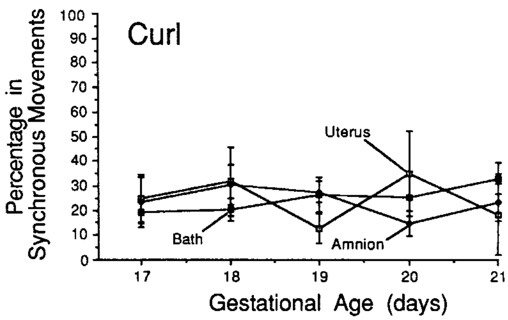


Figure 1. Mean number of categories of movement (±SEM) in the behavioural repertoire of rat fetuses as a function of gestational age and preparation.

overall fetal activity (Smotherman & Robinson 1986). Although synchrony was more common than predicted on day 18 and thereafter, it is still parsimonious to expect that the most abundant categories of synchronous movement will be those consisting of the most commonly moved body regions. To make this expectation more explicit and precise, we used a stochastic model to generate expected frequencies in nine focal categories of synchronous movement (eight types of synchrony and one lumped category, OTHER) for fetuses of five ages (days 17–21) observed in each of the three environmental conditions (In Uterus, In Amnion, In Bath). These expected values were then compared to the observed incidence of synchronous movements to test the accuracy of such a random model of fetal behaviour. This approach resulted in 135 comparisons in which the model was tested (Table I).

Discrepancies between expected and observed frequencies of particular categories of synchronous movement were found; 39 of 135 comparisons (29%) revealed significant deviation from the model. Differences were apparent, in at least two comparisons, for seven of the nine focal categories. Deviation from the random association model was most common among subjects observed In Bath (21 of 45 comparisons = 47%), slightly less common In Amnion (15 comparisons = 33%), and least common In Uterus (three comparisons = 6%). However, there did not appear to be any systematic deviation from the model as a function of fetal age.



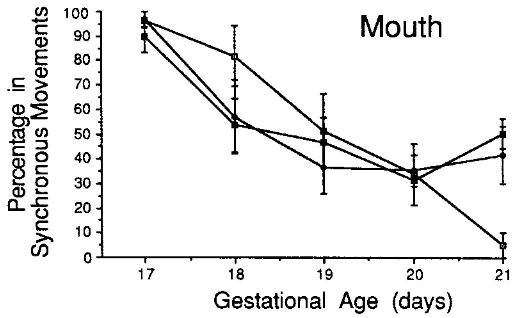
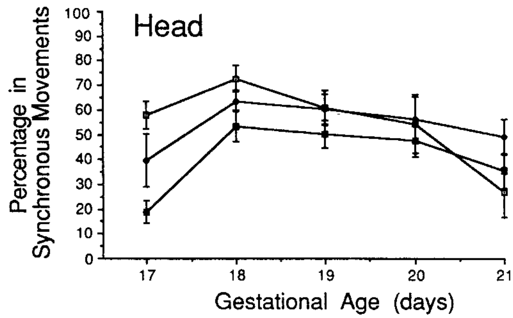
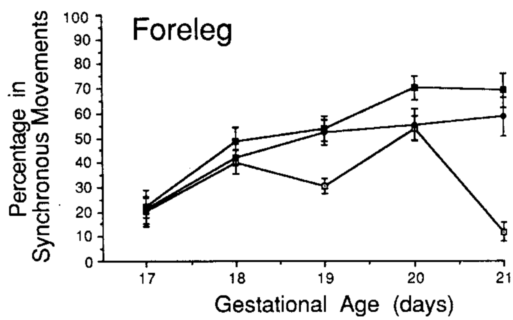


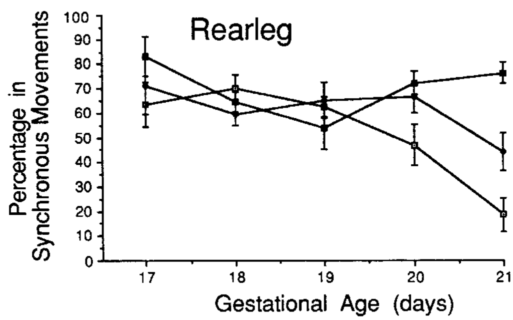
Figure 2. Developmental changes in an index of affinity for five body regions: curl, mouth, head, foreleg, rearleg. Affinity was calculated as the percentage of all movements of a particular region that occurred in synchrony with another region. Points represent mean affinity ± SEM.



The model appeared to be broadly predictive of synchronous movement under most conditions examined (71% of 135 comparisons showed no difference, which was not the result of inadequate sample sizes), and particularly accurate in predicting frequencies of CF and FM.

For six categories of synchronous movement, fetal behaviour could not be accurately predicted by a simple random process. Four of these categories (FH, FR, HM, FHM) occurred with greater frequency than expected, while two categories (HR and FHR) showed inconsistent directions of deviation from expected frequencies. The remaining category, OTHER, which included many movements consisting of two body regions and nearly all movements consisting of three or more body regions, was consistently less frequent than expected. The pattern of results evident in this analysis indicates that, for certain categories, synchronous fetal behaviour is not the product of the random or chance association of different regional movements. In consideration of this finding, subsequent analyses categorized synchronous movements operationally, namely, by considering each unique combination of body regions as a discrete category of behaviour.





Repertoire of Fetal Behaviour

As specified above, our observation protocol provided 127 different categories of fetal movement. This maximum repertoire size, given equal occurrence of each category, can be expressed in terms of a quantitative measure of behavioural

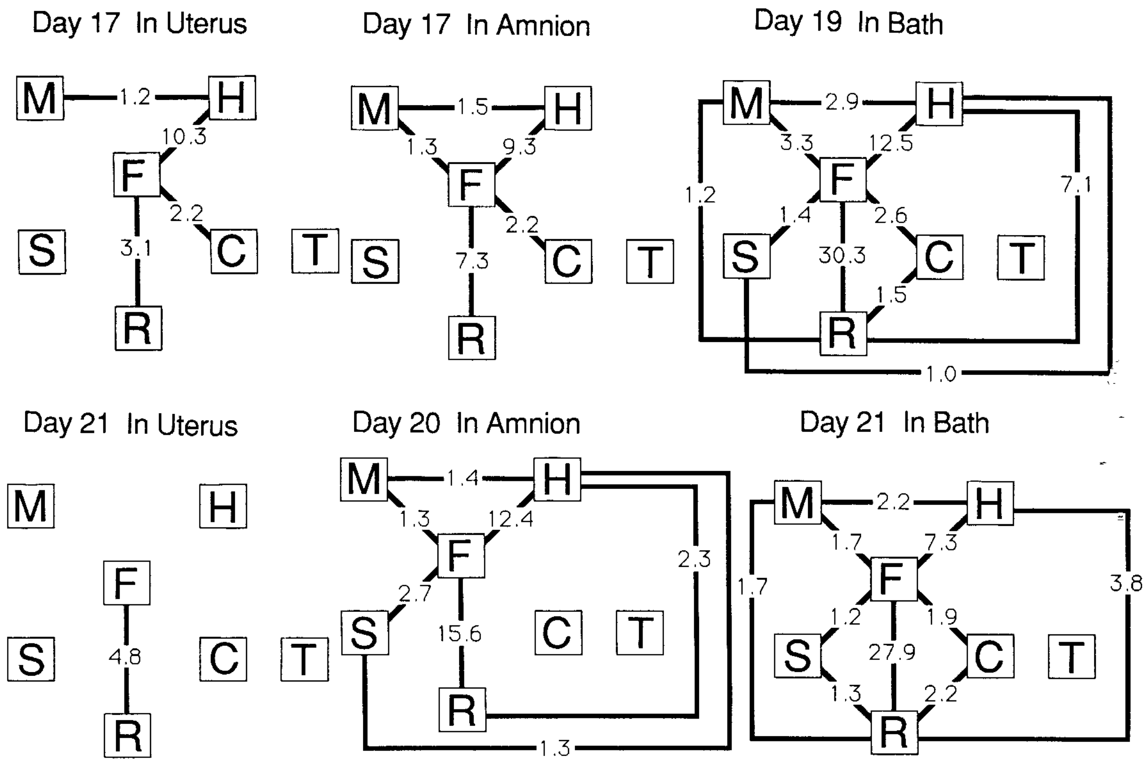


Figure 3. Representative diagrams depicting linkage relationships between pairs of body regions on different days of development. In Uterus, In Amnion or In Bath: M (mouth), H (head), F (foreleg), S (stretch), C (curl), T (twitch), R (rearleg). Each line connects two regions that moved synchronously with a mean frequency exceeding 1·0 movements per 10 min. Numbers superimposed on lines show actual mean frequencies of synchronous occurrence. For example, on day 17 In Uterus each fetus moved foreleg and head synchronously on average 10·3 times during an observation period (including all joint occurrences regardless of other associated regions, such as FH, FHR, FHM, etc.).

diversity: $H_{max} = 6{\cdot}99$ bits/fetal movement. In actuality, individual fetuses exhibited behaviour that varied in diversity from 0·0 bits/movement (day 16 In Bath) to 3·60 bits/movement (day 19 In Bath). This diversity index was calculated for each fetus and subjected to a 6 (age) × 3 (preparation) ANOVA. Significant main effects were evident for both age ($F_{5,192} = 71{\cdot}8$, $P < 0{\cdot}01$) and preparation ($F_{2,192} = 21{\cdot}2$, $P < 0{\cdot}01$). The interaction of these factors was also significant ($F_{10,192} = 6{\cdot}2$, $P < 0{\cdot}01$). Post-hoc tests revealed a general pattern of behavioural diversity that increased from day 16 through day 18 and remained high thereafter. Differences between preparations were apparent at the youngest age, with fetuses In Bath exhibiting less diversity than In Amnion or In Uterus on day 16. On days 18–21, however, diversity In Bath and In Amnion was significantly higher than In Uterus.

The major influence on these diversity changes was the overall repertoire size exhibited by each fetus. Of the 127 possible behavioural categories, 34 were actually exhibited by fetuses, although no individual fetus performed more than 24 categories of movement. To quantify changes in repertoire size, the number of categories observed in each fetus was analysed in a 6 (age) × 3 (preparation) ANOVA. As with measures of diversity, both main effects and the interaction were significant: age ($F_{5,192} = 36{\cdot}6$, $P < 0{\cdot}01$), preparation ($F_{2,192} = 27{\cdot}7$, $P < 0{\cdot}01$), age × preparation ($F_{10,192} = 4{\cdot}27$, $P < 0{\cdot}01$). The pattern of these changes in repertoire size are depicted in Fig. 1. Post-hoc analyses revealed a pattern of difference that was similar to the diversity results, with repertoire size increasing over days 16–18 in all three preparations. On day 18 and thereafter, the size of the repertoire diverged among the three preparations, with the largest repertoire shown by fetuses observed In Bath, intermediate In Amnion and smallest In Uterus.

Affinity between Body Regions

The random association model of synchronous movement, discussed above, failed to predict accurately the relative frequencies of various categories of synchronous movement because different body regions varied in their likelihood of being temporally associated with other regions. An index of affinity was used to express the probability that a particular body region moved at the same time as another body region. Movements in each body region (e.g. foreleg, head, rearleg, etc.) were classified as either single or synchronous. Totals of these two classes of movements for each fetus were used to calculate the percentage of movements of a particular body region that were synchronous. These affinity scores were analysed in a 5 (age) × 3 (preparation) ANOVA. This analysis was applied to only five regions; the relative infrequency of stretch and twitch precluded such analysis. (Smotherman & Robinson 1986 describe the occurrence of stretch and twitch at various gestational ages.)

Variation in the affinity index with age and preparation are depicted for each body region in Fig. 2. Most curl movements occurred in isolation (Fig. 2, Curl) and their tendency to occur alone was unaffected by fetal age or preparation. Mouth movements, on the other hand, virtually always occurred in association with another body region on day 17 (Fig. 2, Mouth), but its high affinity diminished over the next 4 days, as evidenced by a significant main effect of age ($F_{4,101} = 12{\cdot}8$, $P < 0{\cdot}01$). The analysis of head movements indicated significant main effects of both ages ($F_{4,151} = 7{\cdot}0$, $P < 0{\cdot}01$) and preparation ($F_{2,151} = 4{\cdot}8$, $P < 0{\cdot}01$). Head movements occurred more often in association with other regions on days 18–20, but occurred in isolation more often on days 17 and 21. Affinity was generally higher In Amnion and In Uterus than In Bath (Fig. 2, Head).

Two body regions, foreleg and rearleg, were influenced in a more complicated way by fetal age and environmental conditions. The tendency for forelegs to move in synchrony with other regions increased over days 17–21 for fetuses observed In Amnion and In Bath, but decreased on day 21 for fetuses observed In Uterus, as the significant interaction indicated ($F_{8,161} = 5{\cdot}1$, $P < 0{\cdot}01$; Fig. 2, Foreleg). In contrast, rearleg movements were consistent in their likelihood to occur in synchrony over all ages for fetuses In Bath, but affinity diminished slightly In Amnion and more sharply In Uterus on day 21 (age × preparation interaction: $F_{8,149} = 4{\cdot}2$, $P < 0{\cdot}01$; Fig. 2, Rearleg). The different patterns reflected in these analyses suggest that chronological development and sensitivity to environmental influence are unique for each of the five regions of the body considered.

Linkage between Body Regions

A more detailed view of how individual body regions moved in synchrony with other regions was obtained by examining the frequency of temporal linkages between pairs of regions. Linkage diagrams were constructed for each fetal age and preparation to depict patterns of movement synchrony. We have selected representative examples of these diagrams for presentation in Fig. 3. Although there is no simple way to compare these diagrams quantitatively, inspection permits several trends to be identified. Most generally, in our attempts to prepare these diagrams we noted they could be conveniently drawn with a minimum crossing of connecting lines when the relative position of the seven body regions corresponded to their physical location in the fetus. Viewed this way, each diagram was drawn with the anterior of the fetus at the top and posterior at the bottom of the diagram. This suggests that the proximity of body regions influences the frequency of synchronous movements.

On day 17 of gestation, fetuses in the three preparations varied little in synchronous movement; the diagrams at this age possess virtually identical patterns of linkage (Fig. 3, day 17 In Uterus and In Amnion). By day 21, however, the three fetal preparations had distinct effects on the incidence of synchrony. Fetuses In Uterus exhibited the simplest pattern of linkage (i.e. the fewest number of connecting lines, Fig. 3, day 21 In Uterus), whereas fetuses In Bath exhibited the most intricate pattern of linkage (Fig. 3, day 21 In Bath). Within a given preparation, age-related changes in the intricacy of diagrams were often apparent (e.g. Fig. 3, days 17 and 21 In Uterus; days 17 and 20 In Amnion). In other instances, the complexity of the diagram remained stable over several days within a preparation (Fig. 3, days 19 and 21 In Bath).

Overall, there was a strong indication of an interaction between age and preparation across the series of 15 diagrams. Fetuses were differentially influenced by the environmental condition and their gestational age at the time of observation.

Examination of particular connected pairs also revealed several consistent patterns of linkage. The two most common linkages were foreleg–rearleg and foreleg–head. Even when rearleg movements were less common, this foreleg–rearleg linkage was noticeable (Fig. 3, day 17 In Uterus and In Amnion). Twitch was isolated in all 15 diagrams, connecting with no other body region. In part, this was due to the relative rarity of twitch movements. But another regional movement, stretch, was also rare yet often linked with movement of the foreleg, rearleg and head after day 19 (Fig. 3, day 20 In Amnion, days 19 and 21 In Bath). Mouth–head movement occurred with a stronger linkage across ages than might be expected from the frequency of the individual regional movements, Mouth and Head. The physical proximity of these two regions, however, suggests that such a linkage should be common. Such a proximity argument is weaker in the case of the linkage of mouth–foreleg, which also occurred with higher frequency than one might expect based on the relative rarity of Mouth movements.

DISCUSSION

We have previously presented evidence that, after day 17 of gestation, rat fetuses begin to perform synchronous movements more frequently than may be accounted for by the chance association of individual body regions (Smotherman & Robinson 1986). In this paper we elaborate on this finding by presenting a more detailed model and description of specific patterns of behavioural synchrony. The inadequacy of a random association model to predict the frequency of certain categories of synchronous movement is further evidence that the behaviour of fetal rats begins to exhibit spatial and temporal organization about day 17 of the 21-day gestation. This conclusion is supported by the finding that certain regional movements are more likely to occur in synchrony and that specific linkages between pairs of body regions can be identified. In contrast to earlier characterizations of fetal movement as random motility (Smotherman & Robinson, in press), the late prenatal period appears to be the point at which true behaviour begins to emerge.

The general finding of emergent behavioural organization in fetal rats broadly agrees with descriptions of motor development in chick embryos, *Gallus domesticus*. From the onset of motor activity on day 4, movement by the chick embryo is spontaneous and rhythmic, but appears jerky and uncoordinated. Behavioural organization begins to emerge on day 17 as coordinated pre-hatching movements become recognizable (Oppenheim 1973; Bekoff 1981). Pre-hatching behaviour and synchronized movements continue to increase as a proportion of total activity through hatching on day 21 (Provine 1980), even though the overall rate of movement declines. During the last 24 h in ovo, discrete action patterns such as head-tucking and pipping appear which are functional in the hatching process.

The degree to which fetal behavioural organization is evident varies with the micro-environment in which the rat fetus is observed. Generally, the behaviour of fetuses observed In Amnion and In Bath exhibits greater organization (more deviations from a random association model, more pronounced affinities and stronger linkages between body regions) than fetuses observed In Uterus. There are two obvious ways to interpret this finding. First, preparation of the fetus by externalization from the uterus may produce artefacts that are misapprehended as behavioural organization. Alternatively, viewing the fetus in various micro-environments may remove environmental limitations, such as physical restraint (Smotherman & Robinson 1986), that ordinarily inhibit the expression of behavioural organization. We favour the latter of these alternatives because (1) it seems improbable that any perturbation of a system would create the false impression of organization in many independent variables, (2) the kinds of behavioural changes associated with fetuses observed In Amnion and In Bath are in the opposite direction (increased activity and diversity, greater temporal and spatial patterning) than those described for moribund fetuses (Windle 1944), and (3) independent evidence suggests the appearance of coordinated action patterns during the last few days of gestation (Bekoff & Lau 1980; Smotherman & Robinson, 1987b).

Emergent coordination is further implied by patterns of linkage between body regions. Certain linkages stand above the background of other synchronous movements as more common than one might expect from the frequency of regional movements or proximity of body regions. The most noteworthy of these are foreleg–rearleg, foreleg–head, foreleg–mouth and stretch with foreleg, rearleg or head. Indeed, striking parallels between these prenatal linkages and early postnatal action patterns can be identified. Although newborn rat pups do not possess an extensive behavioural repertoire, they do exhibit a handful of coordinated action patterns associated with suckling and milk ingestion. Pups can move towards the lactating mother (locomotion), search for and locate the nipple (probing and rooting) and ingest milk (suckling). Locomotion and body orientation involves coordinated movement of forelegs and rearlegs. Probing and rooting involve synchronous head and mouth movements. Suckling is associated with paw-treading, comprising synchronous mouth and foreleg movements. At the moment of milk letdown by the mother, rat pups exhibit a characteristic stretch posture that involves stiffened back, extended forelegs and hindlegs, and raised head (Hall & Rosenblatt 1977). Further, certain forms of chemosensory stimulation elicit facewashing in older pups, which consists of wiping movements of forelegs in contact with the head and mouth (Johanson & Shapiro, unpublished data). We view each of these patterns of behaviour as the product of motor sharpening (Hailman 1967) of prenatal linkages in evidence as early as day 18 of gestation.

Viewed in this way, the transition from prenatal to postnatal behaviour may not be as discontinuous as is often tacitly assumed. In utero, the fetus' capacity for behavioural organization is subject to environmental inhibition. While coordinated behavioural patterns occur infrequently before birth, their rarity may be due only to the lack of appropriate environmental conditions and eliciting stimuli. For example, day-20 fetuses receiving an intra-oral infusion of a novel chemosensory stimulus while In Bath (such as an extract of lemon or mint), perform facewashing movements (Smotherman & Robinson 1987a). In contrast, fetuses infused with milk (a biologically important stimulus) show no evidence of facewashing but do respond with the characteristic stretch posture (Smotherman & Robinson 1987a). These continuities contribute to the growing evidence that fetuses exhibit a diverse behavioural repertoire and are responsive to environmental change.

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