

Proximal Control of Fetal Rat Behavior

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We examined the influence of the amniotic sac on spontaneous movement in late gestation fetal rats. Using techniques for in vivo observation of fetal behavior, Day 21 rat fetuses were exteriorized from the uterus, with umbilical connections to the dam intact, and videotaped for 15 min either: (a) through the intact amniotic membranes, or (b) following removal of the membranes. Analysis of fetal behavior categories replicated the findings of previous investigators: Movements of the head, forelimbs, and rearlimbs were significantly increased by sac removal, as was the total frequency of behavior categories and the simultaneous occurrence of different behavior categories. Frame-by-frame analysis of videotaped behavior revealed that amniotic sac removal increased the frequency of movement bouts without altering the overall amount of time that fetuses spent moving. Movement bout durations ranged from 50 msec to 70 s. The average duration of movement bouts was significantly reduced for fetuses lacking the amniotic sac as compared to fetuses within the sac, as was the overall distribution of movement bout durations. Frequency distributions of movement bout durations and interbout interval (IBIs) revealed that sac removal significantly increased the occurrence of short (1–2 s) movement bouts and reduced the frequency of protracted movement bouts and interbout intervals (> 10-s duration). Taken together, these findings indicate that the quantitative dimensions of fetal rat movements are influenced by proximal features of the uterine environment. During prenatal life, the amniotic sac appears to sustain movement, possibly by providing proprioceptive feedback or physical support to the fetus, or by regulating the chemical milieu. © 1994 John Wiley & Sons, Inc.

Introduction

Embryos of virtually all species exhibit movement prior to birth or hatching (Bekoff, 1976; Coghill, 1929; Hamburger, 1963; Humphrey, 1964; Windle, 1944). Prenatal movements have been classified as either spontaneous, that is, occurring in the absence of obvious eliciting stimuli, or reflexogenic, that is, evoked by sensory stimulation. In many species, there is precocial emergence of efferent relative to afferent processes (Coghill, 1929; Hamburger, 1963; Narayanan, Fox, & Hamburger, 1971). Together with classic demonstrations of the independence of fetal motility from sensory input

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(Carmichael, 1926; Hamburger, 1963), researchers concluded that the prenatal sensory environment played little role in embryonic movement patterns. More recently, however, researchers have challenged this view. First, studies of sensory features of the intrauterine world have shown that fetuses are exquisitely sensitive to stimuli that are present in utero, including those that are available to the chemical and acoustic modalities (Fifer & Moon, 1988; Pederson & Blass, 1982; Vince, Armitage, Shillito-Walser, & Reader, 1982), as well as those sensed by vestibular and proprioceptive receptors (Bradley & Mistretta, 1975; Ronca & Alberts, 1993).

In addition, there is ample evidence that the expression of certain early behavior patterns is dependent upon context, and thus on the sensory environment. Dramatic examples of this context dependency are seen when particular environmental manipulations, such as substrate, temperature, or body position, unmask behavior patterns precociously, that is, considerably before they are normally performed. The appropriate contextual alterations have revealed coordinated stepping in human babies (Thelen, 1986), hindlimb use in frogs (Stehouwer & Farel, 1984), wing flapping in chicks (Provine, 1981), complex grooming movements in newborn mice (Fentress, 1981), scratching in kittens (Bradley & Smith, 1988), and independent ingestion in preweanling rats (Hall, 1979) prior to their normal time of emergence. Conversely, behaviors that are present in early life but disappear with maturity can be reinstated with specific contextual manipulations. For example, stepping movements of human infants normally performed prior to 2 months of age were restored in older infants by placing them in a supine position or by submerging their legs in water (Thelen & Fisher, 1983; Thelen, Fisher, & Ridley-Johnson, 1984). Bekoff and Kauer (1984) restored hatching movements in posthatching chicks by folding them into glass eggs. Facial wiping, a stereotyped action pattern that can be elicited by delivery of chemosensory fluids into the oral cavity, is normally observed in prenatal rats but disappears after birth. Placing neonatal pups in a buoyant fluid medium freed the forelimbs from participation in postural maintenance and thereby enabled the elicitation of facial wiping (Smotherman & Robinson, 1989). Thus, the proximal environment is an important control parameter for the expression of behaviors during development, even during prenatal life (for further discussion, see Thelen, 1988).

The prenatal environment of birds and mammals consists of fluid-filled membranes housed within an egg case or a maternal uterus. Gestational changes in this environment, coupled with rapid prenatal growth, may augment the magnitude of stimulation around the time of birth or hatching. Early in gestation, the embryo floats within its amniotic sac, which provides the opportunity for symmetrical growth and movement and may buffer the impact of extrinsic stimuli (Bradley & Mistretta, 1975; Ronca, Lamkin & Alberts, 1993). During the final days of gestation, continued physical growth of the fetus and concomitantly reduced amniotic fluid volume cause offspring to become tightly encased within the amniotic sac (Smotherman & Robinson, 1986; Marsh, King, & Becker, 1963; Tam & Chan, 1977). Amniotic fluid also becomes highly viscous near term (Marsh et al., 1963). In combination, these late gestational changes create an increasingly constrained prenatal environment which, in turn, necessitates increased energy expenditure during fetal movement (Smotherman & Robinson, 1986). The "restraint" hypothesis receives support from studies of spontaneous prenatal movement in both rats and chicks in which there is general agreement that embryonic behavioral activity within the amniotic sac is attenuated compared to activity following sac removal (Bekoff & Lau, 1980; Narayanan et al., 1971; Oppenheim, 1972; Smotherman & Robinson, 1986). Smotherman and Robinson (1986) observed that synchronous movements

of different body parts (also termed "Complex Movements" by these authors) were more frequent when fetal rats were released from the amniotic membranes into a warm saline bath (Smotherman & Robinson, 1986). Stochastic modeling suggested that complex movements were more than random ensembles of individual body movements; they were presumed to show an emergent organization in a less constrained prenatal microenvironment (Robinson & Smotherman, 1987). In this view, the amniotic sac and uterus constitute an "environmental limitation" that hides or rechannels the expression of an inherent behavioral organization.

Under some circumstances, however, the amniotic sac may *promote* the expression of organized behavior patterns. The facial wiping response to intraoral fluid infusion could be readily elicited on Day 20 of gestation in fetal rats tested either within, or externalized from, the amniotic sac. If the amniotic sac was intact, this species-typical action pattern could be elicited 1 day earlier in gestation (Robinson & Smotherman, 1991). These authors argued that the amniotic sac provides "scaffolding" which reduced head movement and thereby promoted paw-face contact during facial wiping. Thus, the amniotic sac facilitated the expression of prenatal behavior patterns. Clearly, the restraint hypothesis does not fully explain the influence of the amniotic context on fetal behavioral organization.

Most previous studies of spontaneous behavior in the fetal rat have been based on movement categories (Narayanan et al., 1971; Smotherman & Robinson, 1986), and have thus emphasized a qualitatively rich prenatal behavioral repertoire. One limitation of such methodologies is that important quantitative dimensions of fetal behavior were often lost. In 1980, Bekoff and Lau used frame-by-frame videoanalysis to demonstrate interlimb coordination in fetal rats. Since that time, a number of studies have employed quantitative approaches in studies of movement in embryos and fetuses (Bradley & Bekoff, 1990, 1992; Robertson, 1988; Robinson & Smotherman, 1988, 1991, 1992; Watson & Bekoff, 1990) or similarly immature mammals (Pellis, Pellis, & Nelson, 1992), providing evidence for behavioral organization and temporal and sequential patterning of early movements.

In the present study, we applied both descriptive and quantitative approaches to analyze the proximal control of overall body movement in rats. We report that removal of the amniotic sac alters the temporal structure of spontaneous movement in fetal rats, and propose that the amniotic sac normally plays a role in the patterning of prenatal movement.

We videorecorded late gestation fetal rats *in situ* through the amniotic membranes, or following removal of the membranes. Videotaped behavior of fetuses was analyzed in two ways: First, we coded *behavior categories*, analyzing the form and frequency of different movement types such as head and limb movements (see Smotherman & Robinson, 1986). This type of categorical analysis is useful for describing what fetuses do, and how often they do it. However, different categories of fetal movement do not occur in isolation from one another, but rather, occur in *bouts*. Analysis of *movement bouts*, periods during which the fetus was engaged in movement, and *interbout intervals*, the intervals between movement bouts, reveal much more than do categorical analyses about the temporal aspects of fetal movement. Thus, we conducted frame-by-frame analysis of *movement bouts* to make precise frequency and duration measures of movement under each test condition. This enabled us to: (a) establish the generality of previous reports of increased fetal movement following amnion removal, and (b) examine the temporal parameters of prenatal movement in rats.

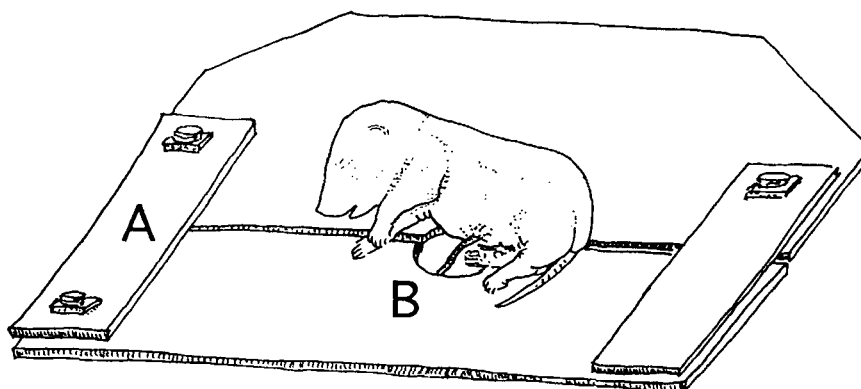


Fig. 1. Fetuses were positioned on a platform constructed from two pieces of plastic and hinged at one end (a) to enable positioning of the surface between the target fetus and litter. A hole (b) in the center of the platform allows passage of the uterine-placental attachment to the dam.

Method

Subjects

Twenty-two adult female Sprague-Dawley rats (70–80d), bred in the Indiana University colony, provided offspring for this experiment. Pregnant dams were housed in standard polyurethane maternity tubs in a colony room maintained at a constant temperature of 22°C, under a 16:8 hr light/dark cycle. Purina Rat Chow and water were available ad lib. Females were time-mated to Sprague-Dawley males. Vaginal cytology was examined each day of the breeding period to identify the date of conception [the first day on which sperm were observed was designated as embryonic Day 0 (E0)]. A total of 22 pregnant rats provided 1 or 2 E21 fetuses ($N = 27$). Seven of these subjects were eliminated from the experiment due to placental separation, behavioral signs of hypoxia, or poor skin coloration (These criteria are described below.) One fetus moved only seven times (for less than 1% of the observation period), and was therefore eliminated from the subject pool. A total of 19 subjects contributed data to this experiment. Animals were maintained and used in accordance with standards for animal care and use as prescribed by the NIH and Indiana University.

Apparatus

For testing, fetuses were positioned on a plastic platform (0.4 mm thick; 8×10 cm) that was partitioned into two equal segments (Figure 1). A hole (Bath condition, 10 mm in diameter; Amnion condition, 20 mm in diameter) was positioned at a central position to accommodate the umbilical-placental attachment to the uterus. For a few subjects ($n = 7$), an experimenter manually supported the platform within the waterbath. For the remainder of the subjects, the platform was stabilized and its height adjusted by attachment to a microscope stand. A gooseneck fiber optics lamp (Reichert-Jung Model 1177) illuminated the field.

Videotapes were made using a color video camera (Sony DXC-107) fitted with a high resolution lens (Schneider-Kreuznach Cinegon 1:1.8/10). The camera was connected to a VCR (Panasonic NV-8950) through a time-date generator (Panasonic WJ-810) with

.01-s resolution. Recording tape speed was 30 frames (60 fields) per second. During playback, videotapes were analyzed frame-by-frame using a VCR with full shuttle/jog control over tape speed in both forward and reverse (Sony AG1960).

General Procedure

Fetal rats were observed using standard procedures for eliminating maternal sensation and voluntary movement in the lower body (Narayanan et al., 1971; Smotherman & Robinson, 1986). Briefly, a pregnant dam was anesthetized with ether and a chemomyelotomy performed to transect the spinal cord between the first and second lumbar vertebrae. The dam was placed in a plexiglas chair, her lower body immersed in an isotonic saline bath ($37.5 \pm .5^{\circ}\text{C}$), and her uterus gently externalized into the bath through a midline incision in the lower ventrum. In order to avoid effects of general anesthesia on fetal subjects, the onset of observation was delayed for 20 min following surgical manipulation of the dam (Smotherman & Robinson, 1986).

Test Procedure

Two E21 subjects were tested from each dam, one from each uterine horn. The uterine wall was incised (10–15 mm) at the antimesometrial border between the second and third fetus at the ovarian end of the horn. The fetus in the third uterine position was externalized from the uterus and positioned laterally on a platform within the waterbath (described earlier) adjusted to a height just overlying the uterus. The platform supported the target fetus and served as a buffer from movements of the dam and siblings.

The camera was positioned vertically over the waterbath at a focal length of 11 cm from the target fetus. Each subject was videotaped at 60 fields per second for 15 min following random assignment to one of two conditions. In the bath condition, the fetus was externalized from the uterus and amniotic sac. In the Amnion condition, the fetus was externalized from the uterus, but not the amniotic sac.

The fetus and its umbilical–placental circulation were closely monitored throughout the observation period and examined at the completion of testing for (a) behavioral signs of hypoxia (repetitive, spasmlike ventroflexions of the trunk), (b) blood loss at the site of placental attachment to the uterus, and (c) pale skin coloration. Each subject was then weighed and its gender determined prior to euthanasia using CO_2 .

Categorical Analysis of Fetal Behavior

Seven basic patterns or categories of fetal behavior observed in rats were coded and analyzed using techniques established by Smotherman and Robinson (1986). These *behavior categories* were: Forelimb, Hindlimb, Head, Mouth, Curl (trunk flexion), Stretch (body/limb extension) and Twitch (spasm in the abdominal region). These categories are sufficient to provide a nearly exhaustive description of behaviors shown by fetal rats (Smotherman & Robinson, 1986). During playback of the videotapes, a microprocessor was time-locked to each subject's record, thereby enabling the scorer to encode the *frequency* of each behavioral category in real time during the 15-min observation interval. This system closely approximates that used by Smotherman and Robinson (1986). Interobserver reliabilities for behavioral coding were determined by

calculating the percent agreement among pairs of scorers for each subject. Average reliability for all comparisons was 95% (range, 84–99%).

Temporal Analysis of Fetal Behavior

For temporal analysis of fetal behavior, movement bouts were coded from videotape by trained observers. A *movement bout* was defined as an interval during which any body segment was in motion, bounded by a minimum of two frames during which no movement occurred. For each subject, the *frequency* and *duration* of each movement bout was measured, and *interbout intervals* (IBIs) were calculated. This was accomplished by reviewing videorecords at normal speed and in slow motion in order to identify the onset and offset of each movement bout to the nearest .01 s. This technique provides temporal resolution accuracy within two fields (.03 s). All of the videotapes were scored by a single observer, then rechecked by two additional observers. Interobserver reliabilities for coding the videotapes were determined by calculating the percent agreement among all scorers for a single subject in each condition. Average reliability for all pairwise comparisons was 96% (range, 89–99%). A total of 2264 movement bouts were analyzed in this study.

Results

All of the fetuses displayed movement bouts that alternated with periods of quiescence. Fetal behavior consisted of displacement of a single body part (limbs, head, or mouth), simultaneous actions of different body parts, or one of the three behavior patterns (twitch, curl, or stretch) described earlier. The quality of fetal movement was variable. Some movements appeared to be undirected and uncoordinated, while others were highly coordinated and distinctive.

Analysis of Fetal Behavior Categories

All fetal behavior was recognizable as one of the seven behavior categories. Fetuses performed an average of 341.9 ± 110.7 movements ($M \pm SE$), at an average rate of 22.9 movements per min. There were no gender differences in movement frequency, male, 326.4 ± 84.7 ($n = 8$); female, 355.8 ± 133.2 ($n = 9$); $F(1,13) = .22$; ns, therefore data for males and female fetuses were pooled for subsequent analyses. A repeated measures ANOVA of overall behavior across 5-min blocks indicated that fetal activity occurred at the same frequency throughout the 15-min test, $F(2,30) = 2.29$; ns.

Overall movement frequency, as measured by the sum of the seven behavioral categories, was greater for fetuses in the Bath condition compared to those in the Amnion group (Table 1). Activities categorized as head, forelimb, and hindlimb movements were significantly greater in fetuses tested in the Bath condition. The frequency of mouth movements, twitches, curls, and stretches did not differ between conditions.

These results suggest that the increase in overall activity associated with removal of the amniotic sac may be due to a selective facilitation of head and limb movements. To examine more closely the composition of fetal behavior in the two test conditions, we corrected for group differences in overall movement frequency by calculating for each subject the percentage of total movement accounted for by each behavior category. Figure 2 illustrates the composition of fetal movement for subjects in each group.

Table 1
Frequency of Behavior Categories in Fetal Rats Observed in Bath and Amnion Conditions

Behavior Category	Group (Mean ± SD)		t test ^a
	Bath	Amnion	
head	91.5 (33.8)	61.0 (23.9)	t = -2.17; p < .05
foreleg	130.6 (29.8)	98.3 (23.7)	t = -2.49; p < .05
rearleg	115.1 (36.7)	83.7 (29.7)	t = -1.95; p < .05
mouth	6.9 (5.9)	10.9 (10.9)	t = 0.93; ns
twitch	12.0 (7.5)	16.6 (10.8)	t = 0.99; ns
curl	35.4 (29.1)	25.3 (17.4)	t = -0.88; ns
stretch	1.6 (1.4)	0.7 (.7)	t = -1.81; ns
total	393.1 (112.0)	296.4 (92.6)	t = -1.95; p < .05

Note. *df* = 15.

Forelimb movement was the most frequent activity, accounting for nearly 40% of all fetal behavior. Movements of other body parts were also frequently observed: Head, forelimb, and hindlimb movement together accounted for over 80% of all fetal behavior. Interestingly, group differences were not obtained for any behavior category. Thus, specific behavior categories do not contribute to the overall increase in movement frequency associated with removal of the amniotic sac. Rather, all behavior categories show a proportionate increase, and the composition of fetal movement is unaltered following sac removal.

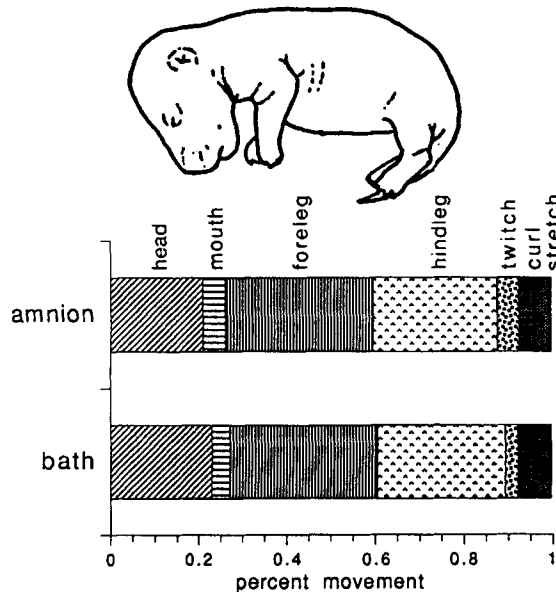


Fig. 2. Frequency of individual behavior categories for fetal rats observed in Bath (n = 8) or Amnion (n = 9) conditions. The frequency of each behavior category is expressed as a percentage of overall behavior.

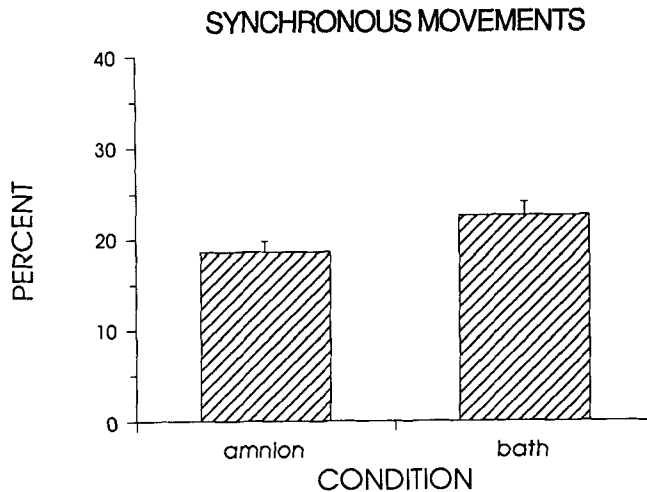


Fig. 3. Percentage of complex movements, expressed as a percentage of overall behavior, displayed by fetal rats in the Bath and Amnion groups.

Synchrony Among Behavior Categories

The number of instances in which different behavior categories occurred simultaneously was determined for each subject from the computer record and expressed as a percentage of the total incidence of behavior categories. Sac removal increased the occurrence of synchronous movements by about 4%, Figure 3; $t(15) = -2.03, p < .05$.

Analysis of Movement Bouts

Movement bout analysis was distinct from the analysis of behavior categories, as discussed in the Methods and the previous Results section. Frame-by-frame analysis of the temporal aspects of videotaped behavior revealed that fetuses in both groups engaged in movement for an average of 5 min, or 31%, of the 15-min observation interval (range, 12–57%). Figure 4 provides a comparative display of parameters of movement bouts for subjects observed in the Bath (left-hand bars) and Amnion (right-hand bars) conditions. The first bar diagram depicts the number of movement bouts displayed by subjects in each group. Fetuses in the Bath condition showed significantly more movement bouts than did those in the Amnion condition $t(17) = -4.71, p < .01$. Nevertheless, overall movement duration was equivalent for the two subject groups, Figure 4, second bar diagram; $t(17) = -0.07, ns$. Intervals between movement bouts were twice as long in the Amnion condition as compared to the Bath condition, Figure 4, third bar diagram; $t(17) = 3.52, p > .01$.

Movement bouts for all fetuses ranged from 0.05–69.19 s in duration. The average duration of movement bouts differed for Bath and Amnion subjects (Figure 4, bar diagram), with fetuses in the Bath condition moving for significantly shorter movement bout durations than those in the Amnion condition, $t(17) = 2.48, p < .05$. Thus, fetuses in the Bath condition initiated more movement bouts than did those in the Amnion condition, but the average duration of movement bouts for subjects in this group was less, and occurred following shorter periods of inactivity.

In order to examine more closely the temporal features of movement, the overall distribution of movement bout durations was compared for the two groups. A Kolmog-

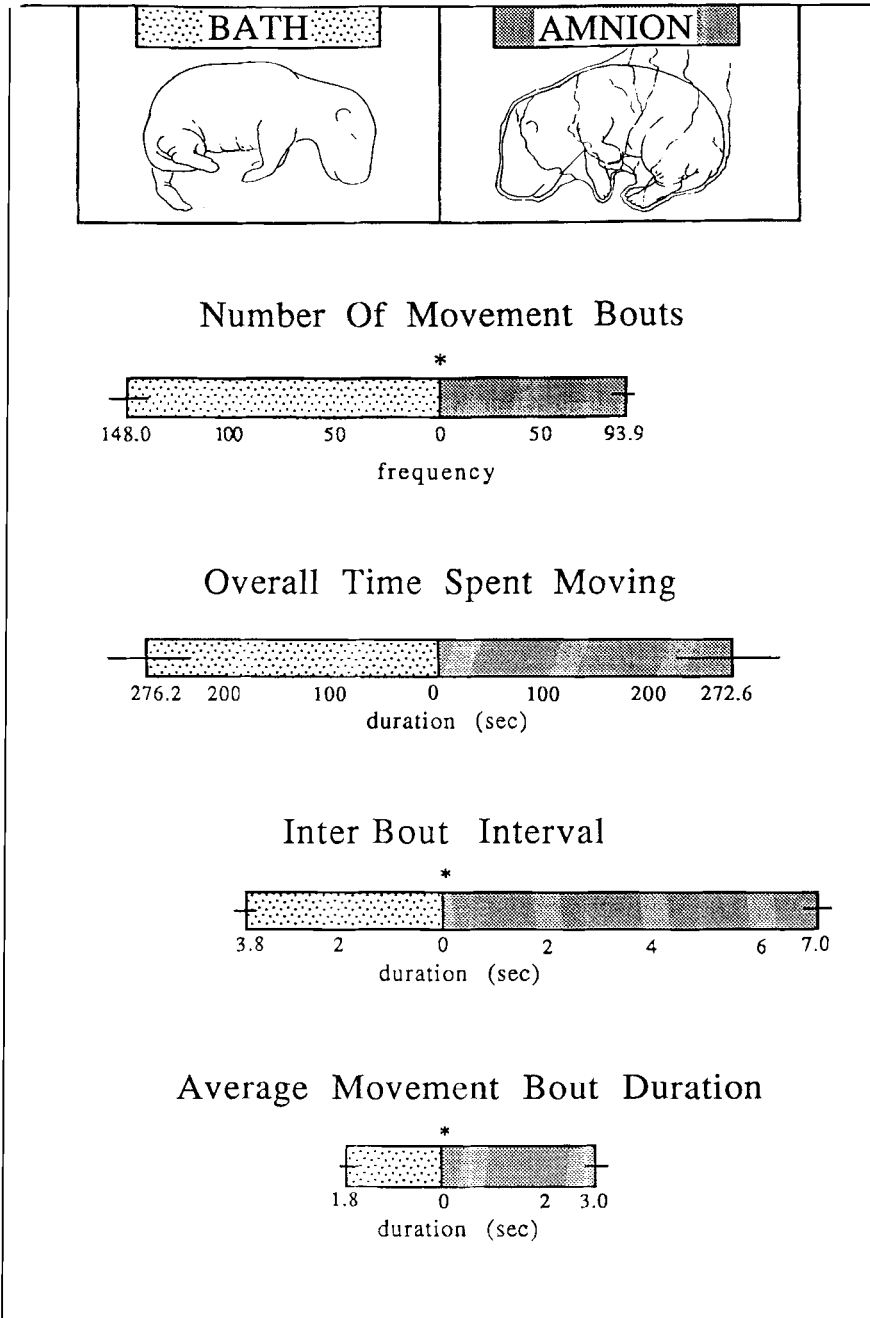


Fig. 4. Temporal parameters of movement bouts for fetal rats observed in Bath (lefthand bars; $n = 9$) and Amnion (righthand bars; $n = 10$) conditions. From top to bottom: Number of movement bouts, Total time spent moving, Interbout interval, and Bout duration.

ovov-Smirnov test revealed that the distributions differed from one another, Bath, $n = 1331$; Amnion, $n = 933$; K-S $X^2 = 15.66$, $p < .05$. In order to identify sources of group differences in the temporal distribution of movement bouts, we constructed frequency distributions of movement bout durations for each subject group (Figure 5a). The leftmost pair of histograms in Figure 5A show that more than 50% of all fetal movement bouts were brief, less than 1 s in duration, regardless of test condition. The second pair of histograms show that removal of the sac significantly increased the frequency of movement bouts in the 1- to 2-s range, $t(17) = 3.39$, $p < .01$. The rightmost histogram pair reveal significantly reduced frequency of movement bouts greater than 10 s in duration, $t(17) = 2.82$, $p < .01$, in fetuses lacking the amniotic sac.

Kolmogorov-Smirnov comparison of the overall distributions of IBIs revealed that the two subject groups differed significantly from one another, Bath, $n = 1322$; Amnion, $n = 923$; K-S $X^2 = 64.82$, $p < .01$. Examination of the frequency distribution of IBIs (Figure 5b) reveals that about 50% of the interbout intervals were less than 3 s in duration. The rightmost pair of histograms in Figure 5b reveals a significant, selective reduction in the frequency of IBIs greater than 10 s in duration, $t(17) = 2.69$, $p < .01$, in fetuses lacking the amniotic sac.

Discussion

Previous studies of spontaneous behavior in the fetal rat consisted mainly of descriptive accounts, (i.e., approaches that relied on behavior categories for the primary unit of measurement), even when quantitative measures have been made (Robinson & Smotherman, 1987, 1988, 1992; Smotherman & Robinson, 1986). In the present study we made multiple measures, including those derived from frame-by-frame analyses of videotaped movement bouts. Our comparison of these measures in fetuses within and without the amniotic sac makes it possible to consider and compare aspects of the form, frequency, and duration of fetal activities. The picture that emerges from the present, multifaceted analysis has some features that resemble past reports, as well as new patterns and dimensions that make the overall impression more complex and challenging to understand.

Forms of Fetal Behavior

The general patterns of behavior that we observed in the present study were similar to those described by previous investigators in rats (Bekoff & Lau, 1980; Narayanan et al., 1971; Smotherman & Robinson, 1986) as well as chick embryos (Hamburger, 1963; Hamburger & Oppenheim, 1967). Fetal behavior was rhythmic, with bouts of activity alternating with periods of quiescence, and can generally be described as either fast and uncoordinated, or slow and smooth. (For further discussion of this work, see Bekoff, 1988).

The seven behavioral categories established by Smotherman and Robinson (1986) provided a comprehensive and inclusive profile with which to categorize various movement forms displayed by fetal rats. It was possible to recognize and classify movements of discrete body parts (e.g., head, mouth, hindlimb) as well as to recognize more general postural movements (e.g., stretch). In accord with earlier work (Robinson & Smotherman, 1987, 1988; Smotherman & Robinson, 1986), there were no differences in these forms of movement as expressed in Bath and in Amnion conditions. That is,

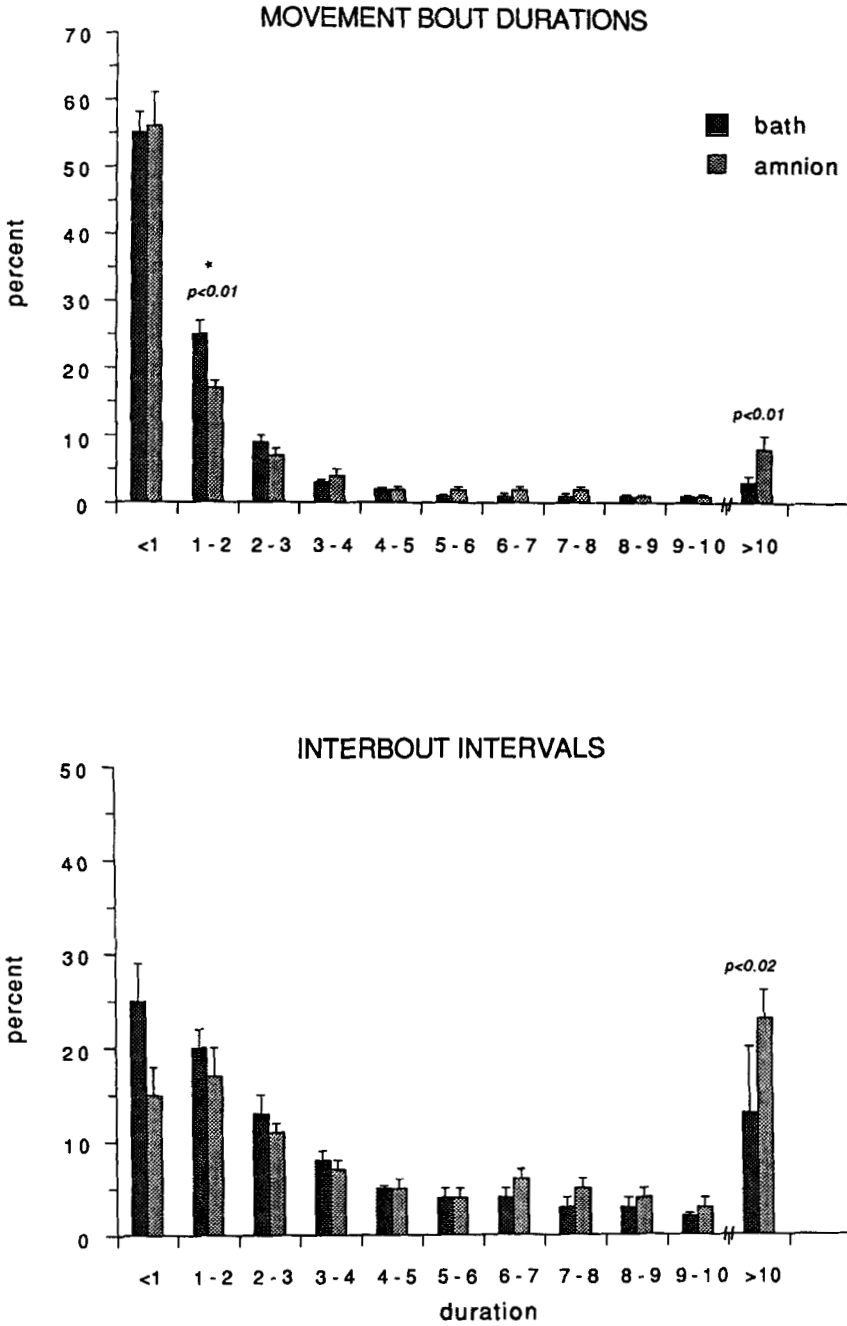


Fig. 5. Bout duration profiles and interbout interval profiles for fetal rats observed in Bath and Amnion conditions. (a) Frequency distribution of bout durations. (b) Frequency distribution of interbout intervals. For each group, data are sorted into 1-s bins and expressed as a percentage of overall movement.

the same movement categories were observed under the different contextual conditions; none were lost or gained with removal of the amniotic sac.

Frequencies of Fetal Behavior

Overall fetal activity, based on categorical data, was somewhat higher in the present study than that reported by Smotherman and Robinson (1986) for 21-day rat fetuses (movements per min: Bath, 26 vs. 15; Amnion, 20 vs. 9). One difference between these two studies is that our subjects were supported on a platform during observation, whereas Smotherman and Robinson (1986) observed fetuses suspended by their umbilical cords in the waterbath. The platform may stabilize fetuses, and thereby promote movements of the head and limbs. This interpretation is supported by a close match between our data and that reported by Narayanan et al. (1971), who also observed their fetuses on a platform.

In accord with previous work (e.g., Smotherman & Robinson, 1986; Narayanan et al., 1971), we found that the frequency of head and limb movements increased significantly following sac removal. Other movement categories (mouth movements, twitches, and stretches) were unaltered in Bath fetuses. We did not observe the significant increase in curls in Bath subjects reported by Smotherman and Robinson (1986). These findings agree with earlier reports of increased movement frequency following removal of the amniotic sac in fetal rats (Bekoff & Lau, 1980; Narayanan et al., 1971; Smotherman & Robinson, 1986).

Figure 2 depicts the overall pattern of fetal behavior, based on categorical data, within the amniotic sac and following sac removal. When the relative frequencies of behavior categories are presented as a *proportion of overall activity*, there are similar percentages of occurrence for each behavior type for Bath and Amnion subjects. Thus, sac removal did not alter the overall composition of fetal behavior, only the absolute frequency with which behaviors were emitted. The fetal behavioral profile, that is, the proportional composition of behavior, was unchanged by sac removal.

Durations of Fetal Behavior

Frame-by-frame videographic techniques enabled us to specify precise temporal parameters of movement bouts. Paradoxically, this analysis revealed that fetuses spent the same amount of time engaged in activity (about one-third of the observation period) whether in the Amnion or Bath condition. That is, even though removal of the amniotic sac increased movement frequency of fetuses, measured by either behavioral categories (Table 1) or movement bouts (Figure 4), the percentage of time spent moving was equivalent in the two conditions. This paradox can be resolved with a comparison of bout duration characteristics exhibited by fetuses under the two conditions. Compared to Amnion subjects, Bath subjects showed a 38% reduction in movement bout duration. Sac removal produced a selective increase of movement bouts within the 1- to 2-s range and a concomitant decrease in bouts exceeding 10 s in duration. Sac removal produced a reduction in the duration of interbout intervals by a similar proportion, with a decline in the proportion of quiescent periods exceeding 10 s in duration. Consequently, the Bath subjects were scored as moving more frequently although the total time they spent moving did not increase because their movement bouts and IBIs were generally short. Thus, removal of the amniotic sac influences the amount of time spent per movement,

but not the amount of time spent moving. Taken together, these results support the view that the sensory environment plays a role in the patterning of prenatal movement.

Implications for the Organization of Prenatal Behavior

Behavior organization in embryos or fetuses is not evident on a purely descriptive level (Bekoff, 1988). Nevertheless, a variety of quantitative and analytic techniques have been used to reveal prenatal behavioral organization (Bekoff, 1976; Bradley & Bekoff, 1990, 1992; Robinson & Smotherman, 1987, 1988, 1991, 1992; Smotherman & Robinson, 1986). Robinson and Smotherman (1987, 1988) applied a stochastic model to movements of discrete body parts of fetal rats. Between Days E18 and E21, specific combinations of these movements co-occurred with frequencies well above chance levels. Robinson and Smotherman (1987, 1988) interpreted these synchronies as evidence of active organization in fetal behavior, and applied the term *complex movement* to fetal activities that involve such joint occurrences of separable movement categories. Sac removal increased the occurrence of these synchronized movements, which they interpreted as an effect due to an altered intrauterine environment. During late gestation, amniotic fluid volume decreases (Marsh et al., 1963; Tam & Chan, 1977) while the fetus becomes increasingly large and occupies progressively more space, rendering the uterine habitat an increasingly restrictive space (Smotherman & Robinson, 1986). Robinson and Smotherman (1987) argue that removing the amniotic sac eliminates these environmental limitations that reduce movement frequency and preclude the expression of complex fetal behavior. They also suggest that movements within the amniotic sac and uterus require increased energy expenditure. Overall movement frequency is thus depressed, as is the performance of complex movements.

The idea that the uterine environment presents proximal stimuli which control fetal behavior is appealing. The results described in the present report are consistent with this basic idea, as are the data of others discussed throughout this article. A variety of hypotheses can be derived from the assertion that fetal behavior is regulated by proximal stimuli, and some of these hypotheses can be examined critically in light of the present findings.

The augmentation of movement frequency in Bath subjects supports the hypothesis that physical restraint in utero necessitates increased energy expenditure during fetal movement, but other considerations do not. Most damaging to this hypothesis is the finding that total movement duration is unaffected by amniotic sac removal. A second source of doubt is that temporal features of fetal movement are differentially affected by the presence and absence of the amniotic sac. Sac removal produced a selective increase in movement bouts lasting 1–2 s in duration and a decrease in protracted (<10 s) movement bouts. If removal of the sac simply reduces the energetic cost of moving, one would predict that bout duration should increase similarly across different intervals of movement. This prediction was not realized.

The outcome of this study, that is, the dissociation between movement frequency and duration, indicates a need for overall caution in interpretations. For instance, the increased incidence of short duration movements in Bath subjects increases the likelihood that their various movements may be scored as occurring jointly, and thus misconstrued as coordinated co-occurrence. We suggest that it is premature to accept the view that fetal movement is complex. It is paradoxical, for example, that complex movements increase with amniotic sac removal (Robinson & Smotherman, 1987, 1988) yet specific, coordinated actions such as facial wiping decrease with the same manipulation (Smoth-

erman & Robinson, 1991). One would predict that the presence of the amniotic sac might either reduce or promote behavioral organization, but not exert both effects.

At this stage of analysis, we suggest some alternative, or additional hypotheses to account for the effects of sac removal on fetal behavior. For example, removal of the amniotic sac may expose fetuses to unidentified stimuli within the artificial waterbath, for example, tactile effects of water movement or contact with particulate matter within the bath. Alternatively, the amniotic sac may exert its effect on fetal behavior by regulating the embryonic milieu. Removal of the sac would thus disrupt the regulation of intraamniotic chemical, pressure, and/or volumetric factors which may, in turn, elicit increased fetal behavior. Or, disruption might be produced via chemosensory or trigeminal stimulation associated with the entrance of the artificial fluid medium into the lungs during fetal breathing or into the GI tract during fetal swallowing. Finally, the amniotic sac may normally prolong individual movement sequences by providing proprioceptive feedback to the fetus. Removal of the sac would remove this source of proprioceptive input, and thereby reduce the incidence of protracted movements produced by fetuses. This possibility receives strong support from the pattern of results that emerged from our temporal analysis: The average duration of movement bouts in fetuses tested without the amniotic sac was significantly longer than those tested within the sac. In addition, comparison of the entire distributions of movement bouts for each group revealed significantly longer movement bout durations in the Amnion group.

Taken together, our results indicate that the total amount of motor output is unaffected by sac removal, but that the *patterning* of movement bouts changes dramatically. It is impossible to determine at this time which of many factors accounts for the effects of sac removal, however each possibility is empirically testable.

Notes

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