Techniques for Observing Fetal Behavior *in Utero:* A Comparison of Chemomyelotomy and Spinal Transection

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Two procedures–chemomyelotomy and spinal transection–have been utilized to chemically block or physically sever the spinal cord of pregnant rats, enabling direct observation of fetal behavior without the use of anesthesia. The effect of each procedure on fetal behavior was studied at four gestational ages (Days 17, 18, 19, and 20). Substantial differences in fetal activity were found between chemomyelotomy and spinal transection groups, including differences in the overall level of activity as well as the pattern of developmental change of behavior. Such differential effects indicate that chemomyelotomy and spinal transection are not equivalent maternal preparations in the study of fetal behavior.

The behavior of fetal rats *in utero* has been studied to describe the prenatal development of behavior (Angulo y Gonzalez, 1932; Narayanan, Fox, & Hamburger, 1971), to investigate the ontogeny of interlimb coordination (Bekoff & Lau, 1980), to probe the ability of fetuses to form learned associations (Smotherman, 1982a,b), and to evaluate the effects of drugs administered prenatally (Kirby, 1979, 1981; Kirby & Holtzman, 1982; Narayanan, Narayanan, & Browne, 1982). Several techniques have been developed to observe fetuses *in utero.* In one general approach, a pregnant female is anesthetized late in gestation, the uterine horns externalized through a midline laparotomy, and the fetuses viewed or manipulated through the uterine wall (e.g., Blass & Pedersen, 1980; Stickrod, Smotherman, & Kimble, 1982). This technique is simple, and because the mother is not permanently impaired, nor the uterus damaged, the fetuses may be allowed to continue development, thereby enabling postnatal testing. However, this approach may not be ideal for observing spontaneous behavior of fetuses. Clear observation of behavior can be obstructed when fetuses are viewed through the uterine wall, and the general anesthesia administered to the mother depresses fetal activity (Kirby, 1981).

To circumvent the effects of general anesthesia, the spinal cord can be severed or blocked in the thoracic region of the pregnant female, thereby eliminating afferent

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stimuli from the lower body. Spinal anesthesia can be achieved by either physically transecting the spinal cord (Kirby, 1979, 1981; Narayanan, Fox, & Hamburger, 1971) or performing a chemomyelotomy by injecting ethyl alcohol into the spinal cord (Basmajian & Ranney, 1961; Narayanan et al., 1982). Both procedures produce permanent paralysis, and fetuses can be delivered outside the uterus for clearer observation. To simulate normal uterine conditions, the entire preparation should be immersed in an isotonic saline solution.

Spinal transection and chemomyelotomy are presumed to have no effect on fetal behavior and have been considered functionally equivalent. However, the two procedures have not been explicitly compared. In this report, we attempt to evaluate the relative merits of spinal transection and chemomyelotomy as techniques enabling the direct observation of fetal behavior *in utero*.

Methods

Subjects

Forty-two adult female Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) were bred to Long-Evans males to produce the fetuses observed in this study. Vaginal smears were taken daily during 4 days of breeding to identify the day of conception; the first day in which sperm were detected was defined as Day 0 of gestation. Throughout pregnancy, females were housed in polycarbonate cages (32.7 x 37.8 x 9.5 cm) in groups of three per cage. Cages remained in a temperature- and humidity-controlled colony room, exposed to a 12/12 hr light/dark cycle (lights on at 1100). Food and water were available ad libitum. Late in gestation—on Day 17, 18, 19, or 20—individual females were selected for either a chemomyelotomy or a spinal transection in preparation for behavioral observations.

Surgical Preparations

Each female selected for surgical preparation was placed in a glass dessicator jar, suffused with ethyl ether, until fully anesthetized. The female was then then laid on a surgical board and a portion of her back and lower abdomen shaved with clippers. Additional ether anesthetic was administered via a nose cone as necessary during surgery.

Chemomyelotomy. The procedure we adopted was similar to those described by Basmajian & Ranney (1961) and Narayanan et al. (1982) to produce an irreversible spinal paralysis posterior to the site of injection. A small longitudinal incision (1 cm) was made through the skin to expose the dorsal musculature in the T12–L1 region of the spinal column. A 25-gauge hypodermic needle was inserted between two vertebrae, the tip being driven laterally and nearly perpendicular to the spinal column; and .05 ml of 100% ethyl alcohol was injected into the vertebral foramen. This procedure was then repeated from the opposite side of the spinal column to insure complete spinal paralysis. A successful preparation was indicated by an immediate extension of the hind legs, followed by several minutes of hyperventilation. Following the chemomyelotomy, the dorsal incision was closed with stainless steel wound clips.

Spinal transection. Narayanan, Fox, and Hamburger (1971) and Kirby (1979) described two slightly different procedures for physically transecting the spinal cord, producing permanent paralysis. We developed a third variant of this method, which involved a small incision through the skin and dorsal musculature in the T11–L1 region of the spinal column. A slender dissecting needle, with the tip bent at an angle of 90°, was inserted through the incision between two vertebrae into the spinal cord. Under tactile guidance, the tip of the needle was then inscribed around the interior circumference of the vertebral foramen to insure complete transection of the cord. As with the chemomyelotomy, spinal transection produced an immediate extensor response of the hind legs followed by hyperventilation. After transecting the cord, the dorsal incision was closed with wound clips. In contrast with the techniques described by Narayanan, Fox, and Hamburger (1971) and Kirby (1979), this procedure required no removal of dorsal musculature or of portions of the vertebral column, and produced little or no blood loss.

Preparation for observation. Following either chemomyelotomy or spinal transection, a midline laparotomy was performed to expose the uterus. The female was secured in a Plexiglas holding device and her hindquarters and abdomen immersed at a 45° angle in a temperature-controlled water bath (37.5°C) containing an isotonic saline solution (Locke's solution; Galigher & Kozloff, 1971). Both horns of the uterus were externalized through the abdominal incision and remained completely submerged in the solution. Wound clips were used to partially close the incision snugly around the uterus, to keep the intestines in place. The fetus nearest the ovary in each uterine horn was then delivered, with care taken to preserve the integrity of the placental-uterine attachment and the amniotic sac, and it was allowed to float freely in the water bath. The mother and fetuses were allowed to recover from anesthesia and acclimate to the water bath for 20–30 min before the first behavioral observation.

Behavioral Observations

We observed fetuses from 42 females in this study: 20 females prepared by chemomyelotomy (five each on Days 17, 18, 19, and 20 of gestation), and 22 by spinal transection (five each on Days 17 and 19; six each on Days 18 and 20). We focussed on the behavior of the two fetuses delivered from the ovarian end of each uterine horn, thus providing a total sample of 84 fetuses observed. Observations were divided into six 5-min periods, alternating between subjects, for a total of 30 min observation per preparation (15 min per subject). The condition of each fetus and the placental-uterine attachment were monitored and subjectively rated at the beginning and end of the observation session. Two persons cooperated to observe and record fetal behavior during a session. One person observed the subject fetus, sorted ongoing spontaneous behavior into predefined categories, and verbally called out the behavioral events. The second person translated the called events into a written record and monitored the temperature of the water bath, elapsed time, and the condition of the mother. To provide an approximate measure of elapsed time and the temporal distribution of activity, the recorder also divided each 5-min observation period into 15-sec intervals (an arbitrary but convenient period). This system enabled the observer to concentrate solely on the behavior of the subject fetus without the distractions of record-keeping, while producing a complete sequential record of behavioral events during each 5-min period.

Seven exclusive and exhaustive categories of spontaneous fetal activity, and one category of maternal activity were distinguished to reflect discrete head, leg, or whole-body movements. Nearly all movements were discrete events; the few instances of continuous activity were divided into a series of events, with each comprising a single vector or cycle of motion of a body part (e.g., an opening and closing of the mouth, or a flexion of a forelimb and return to the initial limb position). Because the categories were defined independently, several events could occur at the same time; thus complex patterns of activity were described as the concatenation of several simultaneous events. While not identical to the behavioral categories used in earlier studies of rat fetuses (e.g., Kirby, 1981; Narayanan et al., 1971; Narayanan et al., 1982), the categories of the present study preserve equivalent information and can be recombined for purposes of comparison. The eight behavioral categories were defined as follows:

1. Head: Any discernible movement of the head, whether dorsal-ventral, lateral, or rotary, relative to the body.

2. Mouth: An opening and closing of the mouth, with or without movement of the tongue (Fig. 1).

3. Foreleg: Any flexor or extensor movement of one or both forelegs originating at the shoulder, elbow, wrist, or digits.

4. Hindleg: Any flexor or extensor movement of one or both hindlegs, originating at or distal to the pelvis; or a movement of the entire pelvic region without twisting or lateral bending of the trunk.

5. Twitch: A momentary, spasmodic movement along the trunk, sometimes involving a violent jerk of the whole body.

6. Curl: A body movement involving a lateral flexion or torsion of the trunk. Curls were distinguished from hindleg movements in causing the posterior end of the body to move to one side of the median sagittal plane (Fig. 2).

7. Stretch: A body movement involving an extension of the trunk, causing the back to straighten or curve slightly and the pelvic region to extend above the horizontal plane of the body.

8. Mother Active: Any movement of the upper body or forelegs of the restrained female rat that caused the uterus and fetuses under observation to be passively moved.

Fig. 1. Photograph of 20-day-old fetus engaged in Mouth and Foreleg movement (Complex activity). Fetuses were clearly visible through the amniotic sac in both procedures and at all gestational ages.

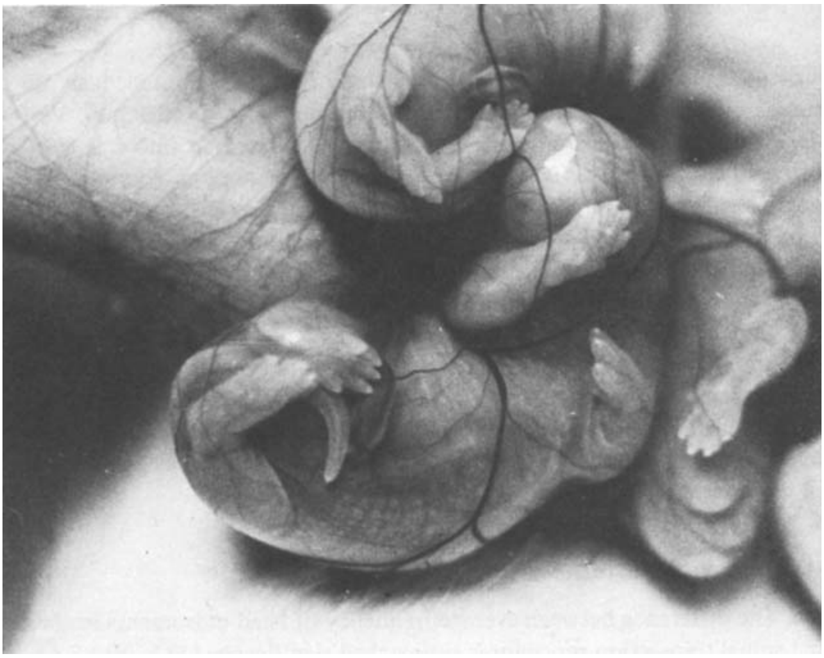

Fig. 2. Photograph of 20-day-old fetus showing Foreleg movement and Body Curl.

Data Analysis

For purposes of analysis, four additional categories were derived from these eight basic patterns of fetal behavior to reflect simultaneous events and overall activity of the fetuses. These derivative categories were as follows:

1. Complex Activity: Two or more behavioral events of a fetus that occurred simultaneously (e.g., Foreleg and Hindleg).
2. Total Whole Activity: The total number of times a fetus was recorded as active, regardless of whether it performed single or complex behavioral patterns, in a 5-min observation period. Each complex event, involving several simultaneous actions, was scored as a single bout of activity.
3. Total Component Activity: The total number of behavioral movements of a fetus in a 5-min observation period. Component Activity differs from Whole Activity in the way complex actions are scored; each behavioral component in a simultaneous complex event (e.g. Foreleg, Head, and Curl) is counted separately.
4. Periods of Inactivity: The number of 15-sec intervals, recorded during a 5-min observation period, in which no fetal activity was noted.

Frequency counts of events in the eight basic categories and four derivative categories were subjected to three-way ANOVAs for statistical analysis. Frequency counts were also converted to percentage scores (frequency of event/total component activity) to examine the relative frequency of different behavioral patterns. An alpha level of $p < .05$ was used to judge significance in all statistical analyses.

Results

All of the fetal behavior observed in this study occurred spontaneously, in contrast to responses evoked by external stimulation (e.g., pinpricks or stroking). Very little of the observed behavior appeared well coordinated or integrated into a larger pattern of behavior; virtually all fetal activity involved individual or random combinations of complex trunk, head, or leg movements. However, we noted two exceptions to this overall pattern of unintegrated activity. On occasion, fetuses moved one or both forelegs synchronously along the side of the mouth, toward the nose, sometimes as the mouth was opened and closed. Such wiping movements were rare, and were lumped with all other foreleg movements in this analysis. A second, infrequent exception was the coordinated movement of left/right or fore/hind pairs of legs either in synchrony or alternation. These movements resembled the locomotor movements of young rat pups. Generally, coordinated leg movements were short in duration and associated with other uncoordinated leg movements; we have considered them together with all other foreleg and hindleg movements in this analysis.

Fetal Behavior Patterns

Head. The difference between average frequency of head movements in chemomyelotomy and spinal transection procedures approached significance ($F(1,76) = 3.65$, $p = .059$; Fig. 3). No main effect of fetal age was apparent ($F(3,76) = 2.00$, $p > .10$). However, a significant interaction between Procedure and Age was found ($F(3,76) = 3.30$, $p < .05$), indicating that younger fetuses show more head movement in chemomyelotomy preparations and that older fetuses show more head movement in spinal transections. When compared on the basis of percentage of total activity, neither of the main effects were significant (Procedure: $F(1,76) = 0.96$, $p > .30$; Age: $F(3,76) = 0.21$, $p > .80$). However, the Procedure × Age interaction was significant ($F(3,76) = 3.14$, $p < .05$.

Mouth. The frequency of mouth movements was very similar in both procedures ($F(1,76) = 0.31$, $p > .05$; Fig. 3) and across all four ages ($F(3,76) = 0.05$, $p > .90$). The interaction between Procedure and Age also failed to reach significance ($F(3,76) = .29$, $p > .80$). No change in this pattern of results was apparent when mouth movements were examined as a percent of total activity (Procedure: $F(1,76) = .68$, $p > .40$; Age: $F(3,76) = .47$, $p > .70$; Procedure × Age: $F(3,76) = .79$, $p > .50$).

Foreleg. Significantly more foreleg movements occurred in the spinal transection procedure than in the chemomyelotomy ($F(1,76) = 6.10$, $p < .05$; Fig. 3). Foreleg activity was also more frequent in younger fetuses ($F(3,76) = 5.55$, $p < .005$. However, there was no apparent interaction between Procedure and Age factors ($F(3,76) = .09$, $p > .90$). When these data were compared as percentages of total activity, the main effect of Procedure disappeared ($F(1,76) = 1.33$, $p > .25$). Younger fetuses (Days 17 and 18) spent a higher proportion of time performing foreleg movements than did older fetuses (Days 19 and 20) ($F(3,76) = 15.41$, $p < .001$). As with the frequency data, there was no significant interaction between Procedure and Age ($F(3,76) = .05$, $p > .90$).

Hindleg. The difference between chemomyelotomy and spinal transection in frequency of hindleg movements was nearly significant ($F(1,76) = 3.47$, $p = .066$; Fig. 3). Unlike foreleg movements, the frequency of hindleg movements did not fluctuate as a function of age ($F(3,76) = 1.84$, $p > .10$). The Procedure × Age interaction also was not significant ($F(3,76) = .89$, $p > .40$). As percentages of total activity, no main effects of procedure ($F(1,76) = 2.15$, $p > .10$) or gestational age ($F(1,76) = 1.84$, $p > .10$) were apparent; nor was the interaction between Procedure and Age significant ($F(3,76) = 2.54$, $p > .05$).

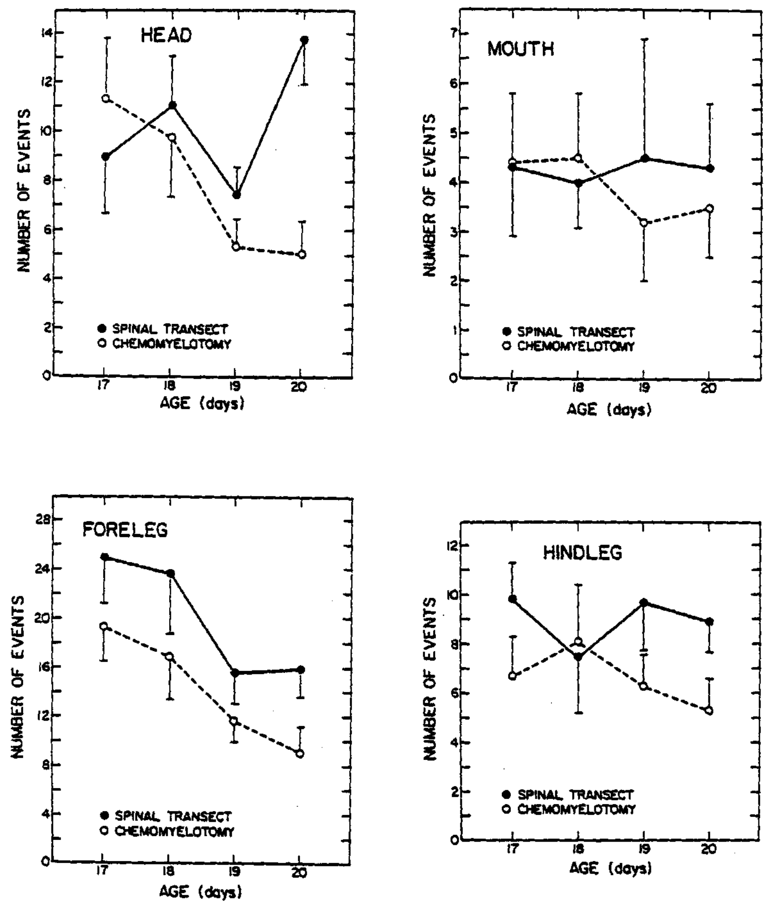

Fig. 3. Mean frequency of four patterns of fetal behavior during a 5-min observation period as related to gestational age: Head movements (upper left), Mouth movements (upper right), Foreleg movements (lower left), and Hindleg movements (lower right).

Body twitch. Body twitches did not significantly differ in frequency between chemomyelotomy and spinal transection ($F(1,76) = 2.06$, $p > .15$). However, there were significant developmental changes in twitches, with older fetuses showing this behavior more often than younger fetuses ($F(3,76) = 7.10$, $p < .001$; Fig. 4). There was apparently no difference in the pattern of developmental change in the two procedures, as evidenced by the lack of an interaction effect ($F(3,76) = 2.08$, $p > .10$). This same pattern of results applies to twitches as a percentage of total activity (Procedure: $F(1,76) = .17$, $p >$ 60; Age: $F(3,76) = 11.39$, $p < .001$; Procedure x Age: $F(3,76) = .76$, $p > .50$).

Body curl. There was no significant difference in the frequency of body curls between subjects treated with chemomyelotomy and spinal transection procedures ($F(1,76) = 2.80$, $p > .05$; Fig. 4). Developmental changes in curling behavior were indicated by a main effect of age, with more curls occurring on Days 17 and 20 than on Days 18 and 19 ($F(3,76) = 3.51$, $p < .05$). The pattern of this developmental change showed no apparent difference between surgical procedures, as evidenced by the lack of a significant Procedure x Age interaction ($F(3,76) = .19$, $p > .90$). There was a significant difference between the two procedures in the proportion of total activity comprising curls, with curls constituting a higher proportion of total activity in chemomyelotomy preparations than in spinal transections ($F(1,76) = 10.84$, $p < .005$). On a percentage basis, the effect of

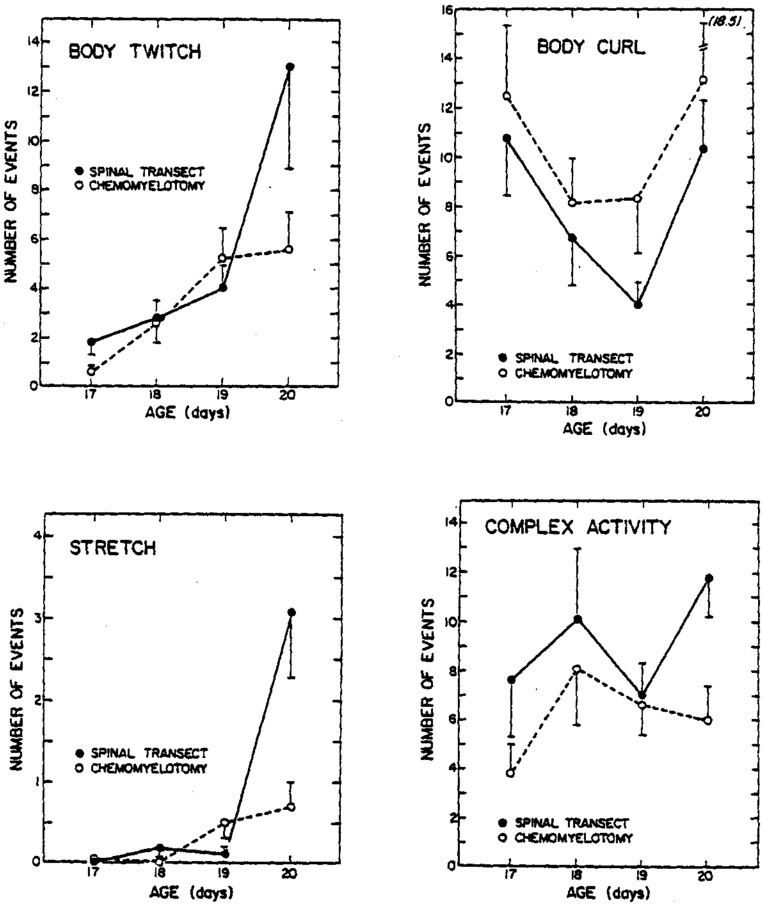

Fig. 4. Mean frequency of four patterns of fetal behavior during a 5-min observation period as related to gestational age: Body Twitch (upper left), Body Curl (upper right), Stretch (lower left), and Complex Activity (lower right).

age disappeared ($F(3,76) = 1.42$, $p > .20$), and the interaction between Procedure and Age was not significant ($F(3,76) = .54$, $p > .60$).

Body stretch. The occurrence of stretching movements appeared to differ markedly between the two procedures, as evidenced by a significant main effect of procedure ($F(1,76) = 17.30$, $p < .001$; Fig. 4). Stretches were rare among 17- and 18-day-old fetuses, but much more common in 19- and 20-day-olds; this developmental effect was significant ($F(3,76) = 17.30$, $p < .001$). The pattern of developmental change in stretching movements was also quite different between the two procedures, with a very large increase in stretches on Day 20 with spinal transections, but only a minor increase on Day 20 with chemomyelotomies, as the significant Procedure x Age interaction demonstrated ($F(3,76) = 8.75$, $p < .001$). When compared as percentage of total activity, the main effect of procedure was nearly significant ($F(1,76) = 3.32$, $p = .072$). Further, the developmental effect ($F(3,76) = 19.31$, $p < .001$), and the interaction between Procedure and Age ($F(3,76) = 6.80$, $p < .001$), were still strongly evident.

Complex activity. On many occasions, fetuses simultaneously moved head, fore-, or hindlegs or combined leg and whole body movements. These complex activities were more common with spinal transections than with chemomyelotomies ($F(1,76) = 5.35$,

$p < .05$; Fig. 4). Across the four ages, complex actions fluctuated in frequency, but no developmental effect was detected ($F(3,76) = 1.63$, $p > .15$). This fluctuating pattern of developmental change apparently did not differ between the two procedures, because the Procedure x Age interaction proved not to be significant ($F(3,76) = .79, p > .50$).

General Fetal Activity

Total activity. Two general measures of overall fetal activity were developed. Whole Activity reflected the total number of bouts of activity during an observation period, whereas Component Activity further divided complex actions into their component head, leg, and body movements. The total Whole Activity of fetuses did not differ between the two procedures ($F(1,76) = 2.98$, $p > .05$; Fig. 5). However, a developmental change in activity was evident, with fetuses showing a decline in total activity from Day 17 through Day 19, then increasing again on Day 20 ($F(3,76) = 2.74, p < .05$). This pattern of developmental change was similar in the two preparations inasmuch as the interaction between Preparation and Age was not significant ($F(3,76) = 1.03, p > .30$).

The same general pattern of activity was seen in Component Actions (Fig. 5). Because complex actions were more common with spinal transections, a difference between the two procedures was evident in Component Activity ($F(1,76) = 4.74$, $p < .05$. However,

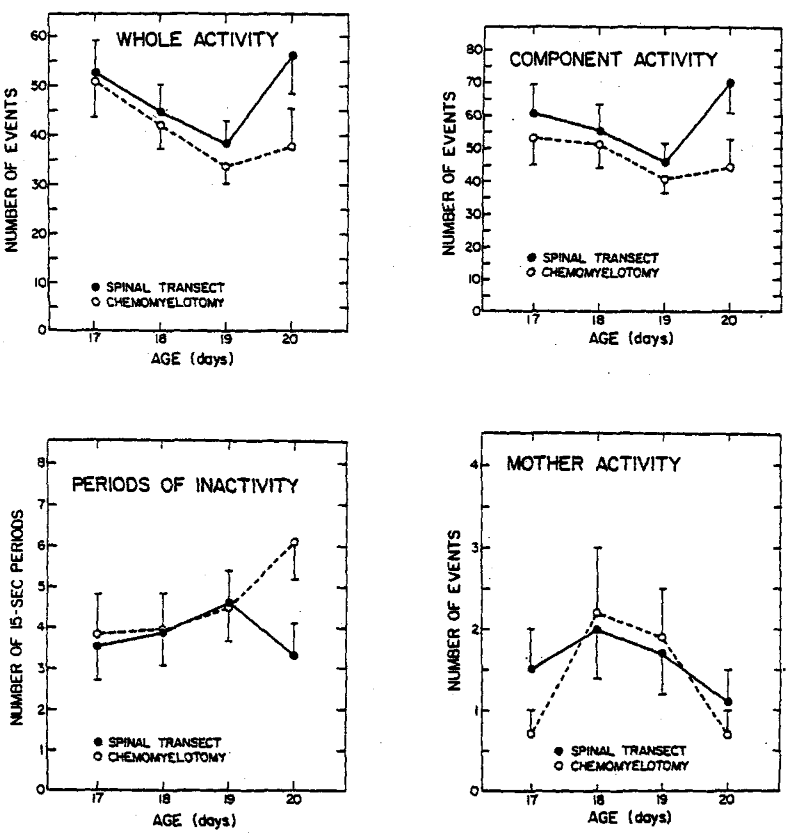

Fig. 5. Mean frequency of three indices of total fetal activity and one of general maternal activity during a 5-min observation period as related to gestational age: total number of behavioral events (upper left), total number of component actions (upper right), number of 15-sec intervals in which a fetus was inactive (lower left), and number of maternal movements (lower right).

developmental changes in activity were not significant ($F(3,76) = 1.81, p > .15$), nor was any interaction between Procedure and Age apparent ($F(3,76) = 1.10, p > .35$). Overall, fetuses were more active in spinal transections than in chemomyelotomy preparations.

Temporal distribution of activity. Fetuses often interspersed bouts of vigorous behavior with periods in which they lay still. To quantify these patterns of activity and inactivity, the number of behavioral events in each 15-sec interval was scored. The number of bouts of fetal activity (Whole Activity) per 15-sec interval ranged from 0 (termed a Period of Inactivity) to 15 in spinal transections and 0 to 13 in chemomyelotomies. With both procedures, the modal number of bouts per interval was 1, but much of the observed activity appeared in bursts or clumps.

To investigate this apparent clumping, the cumulative distribution of 15-sec intervals was plotted as a function of the number of bouts of activity per interval. Such a distribution should approximate a geometric decay function if the occurrences of behavioral events are independent—that is, if the probability of an additional event occurring in an interval is constant and independent of the number of events that have already occurred (Hailman, 1974). As Figure 6 shows, the observed distributions conform to the predicted function, with correlation coefficients of −.991 with chemomyelotomy preparations and −.996 for spinal transections. The least-squares regression lines exhibit different slopes (−.272 for chemomyelotomy and −.197 for spinal transection), indicating that the constant probability of adding another bout of activity in an interval differed between the two preparations. This apparent difference was supported by a chi-square comparison of the two distributions ($\chi^2 = 50.06$, $df = 9$, $p < .001$). When data from the two preparations were broken down by individual day of gestation, no difference was found between spinal transection and chemomyelotomy on Day 17 ($\chi^2 = 3.08, df = 9, p > .90$), or Day 18 ($\chi^2 = 8.71, df = 9, p > .40$). But intervals with many bouts of activity were significantly more frequent with spinal transection than with chemomyelotomy on Day 19 ($\chi^2 = 21.44$,

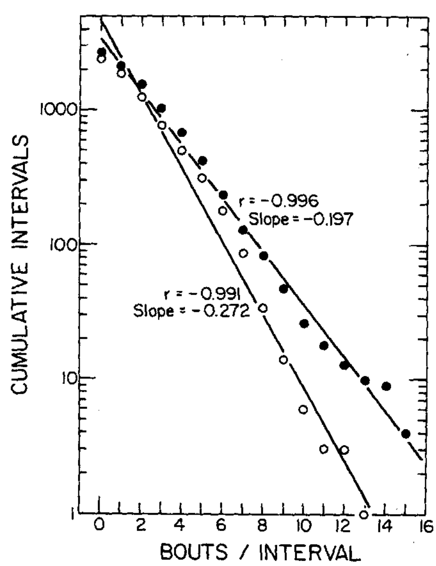

Fig. 6. The cumulative distribution of 15-sec intervals plotted (on a logarithmic axis) as a function of the number of bouts of activity per interval. The distributions for chemomyelotomy and spinal transection both approximate a geometric decay function. However, the slopes of the two least-squares regression lines, shown on the graph, are different, indicating that the probability of adding another bout of activity in an interval is greater with spinal transection than with chemomyelotomy.

$df = 9$, $p < .05$), and Day 20 ($\chi^2 = 63.46$, $df = 9$, $p < .001$). These analyses show that the distribution of activity differed between the two preparations, especially at later gestational ages.

Periods of inactivity. Paradoxically, intervals where fetuses exhibited no movement did not differ significantly between the two procedures ($F(1,76) = 2.27$, $p > .10$), and fetuses showed no general tendency to exhibit more or less inactivity with increasing age ($F(3,76) = .94$, $p > .40$; Fig. 5). The interaction between Procedure and Age was similarly not significant ($F(3,76) = 1.85$, $p > .10$).

Effects of External Conditions

Temporal effects. The effect of elapsed time during a session of observation of fetal activity was assessed by comparing the frequency of behavioral events in each of the three 5-min observation periods. Virtually all behavioral patterns were affected by time; significant changes from the first through the third period were noted, at the $p < .01$ level, for Foreleg, Hindleg, Head, Stretch, Complex, Whole Activity, Component Activity, and Periods of Inactivity, and at the $p < .05$ level for Curls. Twitches also showed a significant effect of elapsed time at the $p < .01$ level when the data were expressed as a percentage of total activity. For all behavioral patterns except Twitch, activity increased in frequency with increasing time in the saline bath. For Twitch, activity showed an initial decrease that leveled off with elapsed time.

Mother activity. Chemomyelotomy and spinal transection procedures might produce differential effects on fetal behavior by inducing different levels of activity in the restrained mother. However, movements by the mother were infrequent, with only one to three maternal movements per 5-min observation period, and maternal activity differed little between the two procedures ($F(1,76) = .28$, $p > .50$; Fig. 5). Maternal activity was also unaffected by gestational age ($F(3,76) = 2.70$, $p > .05$), and no interaction between Procedure and Age was apparent ($F(3,76) = .43$, $p > .70$). Moreover, we could detect no relationship between the frequency of Maternal Activity and total Component Activity of the subject fetus ($r = -.138$, $z = .86$, $p > .30$).

Condition of placental attachment. Each fetus was given a numerical rating (on a scale of 0–2.0) before and after each observation to provide a subjective evaluation of its physical condition and of the condition of its placental attachment to the uterus. Some placentas showed partial separation from the uterus during an observation period, resulting in loss of blood into the water bath. We tested the possibility that fetal condition could have differed between the two preparations, thereby causing different patterns of activity, by examining fetal ratings. No significant difference was found between the ratings taken at the end of an observation session with spinal transections ($\bar{x}$ rating = 1.7) and chemomyelotomies ($\bar{x}$ rating = 1.6, $p > .65$; test for significance of difference between two proportions: Bruning & Kintz, 1968; p. 199). Moreover, total activity did not differ between fetuses with unimpaired placental-uterine attachments and fetuses that showed partial placental-uterine separation ($t = .773$, $df = 72$, $p > .40$).

Discussion

Several studies have described the spontaneous behavior of rat fetuses *in utero,* but direct comparison of different studies is complicated by several factors. Narayanan et al. (1971) employed spinal transection to prepare pregnant females for fetal observation and found that fetal activity increased from Day 17 to Day 18, but their data appear to show a plateau of total fetal activity from Day 18 through Day 20 (mean no. events/5 min Day 17 = 9.7, Day 18 = 18.0, Day 19 = 16.0; Day 20 = 18.7). Kirby (1981), who also used a spinal transection technique, reported more fetal activity on Day 18 than on Days 16 or 21, but calculation of corresponding rates of activity from her data is, unfortunately, not possible. These patterns of developmental change, and especially the absolute levels of fetal activity, appear to differ from the patterns of fetal behavior reported by Narayanan et al. (1982), who relied on a chemomyelotomy procedure. Two different summaries of fetal activity were reported in the latter study, both showing an increase in activity from Day 16 through Day 18 and a subsequent decline through Day 20 (mean no. events/5 min in lower estimate: Day 16 = 90.6, Day 17 = 143.5, Day 18 = 184.7, Day 19 = 142.2, Day 20 = 78.5). We are unable to reconcile the magnitude of the difference between these reported frequencies, especially because the later study (with much higher rates of activity) considered only foreleg, head, and mouth movements, a subset of the total behavioral repertoire of rat fetuses.

The results of the present study fall in between these extremes (Fig. 5). Total fetal activity was near its peak on Day 17, declined through Days 18 and 19, and, in spinal transections, increased again on Day 20. If one considers only foreleg, head, and mouth movements (as in Narayanan et al., 1982), our data also show a peak of activity on Days 17 and 18 and, for chemomyelotomy, a sharp subsequent decline through Day 20 (mean no. foreleg + head + mouth events/5 min: Chemomyelotomy: Day 17 = 34.9, Day 18 = 32.9, Day 19 = 20.1, Day 20 = 17.5; Transection: Day 17 = 38.0, Day 18 = 38.5, Day 19 = 27.8, Day 20 = 33.9). Patterns of developmental change varied among different individual behavioral patterns, however. Body Twitch and Stretch showed a monotonic increase with gestational age; Foreleg showed a monotonic decrease with age; Curl showed high frequency on Days 17 and 20 and low frequency on Days 18 and 19; Mouth showed a fairly constant level of occurrence.

Fetal behavior changed as a function of the length of time fetuses remained in the warm saline solution. Typically, general activity increased from the first 5-min observation period through the third. Several hypotheses might account for our observed increase in activity with time. First, an increase in activity might be ascribed to a slow recovery from the effects of ether anesthesia. This possibility seems unlikely, because (1) ether has transient effects on the behavior of fetuses that disappear only 15-20 min after withdrawal of the ether (Kirby, 1979), and (2) the fetuses observed in this study were allowed to recover for 20-30 min after initial ether administration. Second, a change in activity might be expected if fetuses became deprived of oxygen through placental separation during an observation session. We observed partial placental separation in some but not all fetuses. But no behavioral differences were apparent between fetuses with partial placental separation and fetuses with no separation. In addition, fetuses that exhibited reduced ratings were distributed nearly uniformly among procedures and gestational ages.

The rise in fetal activity with elapsed time in the water bath may reflect a gradual fetal response to greater freedom of movement, rather than to ether or reduced oxygen. Unlike Kirby (1979), who watched fetuses through the uterine wall and reported no temporal changes in activity, we delivered subject fetuses from the uterine horn for observation. This procedure enabled clear observation of fine and subtle movements, but it also removed the fetus from a situation of close physical contact within the uterus. Because large movements were not physically constrained, fetuses may have adjusted to the less crowded conditions with greater activity. Narayanan et al. (1971) also noted that fetuses were more active after being delivered from the uterus, with the placental–uterine attachment intact, than while still within the uterus.

The two surgical procedures used to induce spinal anesthesia in the present study—chemomyelotomy and spinal transection—produced different effects on fetal behavior. The total activity of fetuses was greater with spinal transection preparations than with chemomyelotomy, especially at later ages. Specific patterns of behavior appeared to be more sensitive than others to the kind of procedure used to produce spinal anesthesia. Foreleg and stretch movements, as well as complex actions, were more frequent with spinal transections than with chemomyelotomies. For stretch and complex movements, this difference was greatest on Day 20, while foreleg movement showed a nearly constant difference between preparations across days.

Part of the difference between the two procedures might be expressed as a simple change in overall level of activity: fetuses from spinal transection preparations were generally more active than those from chemomyelotomy. On a finer scale, however, the differential effects of chemomyelotomy and spinal transection appear more complex and nonlinear. For example, head movements showed different patterns of developmental change in the two procedures, tending to increase across days with spinal transection, but decreasing with chemomyelotomy. Stretch movements increased dramatically on Day 20 with spinal transection, but showed a significantly smaller increase with chemomyelotomy. Curls exhibited similar patterns of developmental change with the two procedures, but were more common with chemomyelotomy than with spinal transection, a reversal of the general pattern. As a consequence, curls constituted a higher proportion of total activity in chemomyelotomy than in spinal transection, suggesting that the relationships between different behavioral patterns and the way activity was budgeted was affected differentially by the two procedures. The temporal distribution of activity also differed between procedures, most markedly on Day 20, in a manner consistent with, but irreducible to, simple differences in total frequency of behavior. These findings indicate that the differential effects of the two procedures are not mediated through a general change in the level of overall activity. Rather, each separate behavioral pattern is affected individually in an unpredictable fashion. Generally, differential effects, where present, were most pronounced in older fetuses.

Spinal transection and chemomyelotomy are both powerful techniques that enable direct observation of the behavior of fetuses *in utero*. The results of this study, however, argue that the two procedures are not equivalent in their effects on fetal behavior and should not be regarded as interchangeable. From the present data, we cannot conclude which technique is preferable. Observation of normal fetal behavior *in utero* will always require some kind of artificial manipulation, and it is not yet possible to say which procedure least affects normal fetal activity.

Notes

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