

The Rat Fetus in Its Environment: Behavioral Adjustments to Novel, Familiar, Aversive, and Conditioned Stimuli Presented In Utero

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With the pregnant rat under ether anesthesia, rat fetuses were exposed on Day 17 of gestation to a taste/odor stimulus (mint) injected into the amniotic fluid and/or lithium chloride injected into the peritoneum. Behavior of injected fetuses was directly observed on Day 19 of gestation following chemomyelotomy and laparotomy of the female and immersion of the uterus into a warm saline bath. With these procedures, a series of four experiments was conducted to assess the behavioral effects of (a) the mint taste/odor alone, (b) the LiCl alone, (c) the pairing of mint and LiCl on the day of conditioning, and (d) the reexposure to mint after an earlier pairing of mint and LiCl. These experiments provide clear evidence that rat fetuses are capable of forming conditioned taste/odor aversions as early as Day 17 of gestation and, further, that rat fetuses are capable of expressing these learned aversions in utero.

Several studies recently have shown that manipulation of the uterine environment late in gestation can promote learning by rat fetuses. Rat fetuses that are presented in utero with citral, a lemon scent, prefer to attach and suckle postnatally from nipples scented with citral (Pedersen & Blass, 1982). Similarly, when rat fetuses are exposed to a taste/odor stimulus, such as apple juice, paired with an ip injection of lithium chloride, subsequent experiences with the taste odor will cause the pups to exhibit avoidance behavior. Fetuses exposed to these treatments prenatally, when tested postnatally, (a) avoid nipples painted with the aversive taste/odor stimulus (Stickrod, Kimble, & Smotherman, 1982a), (b) avoid wood shavings scented with the aversive odor (Stickrod, Kimble,

& Smotherman, 1982b), and (c) exhibit longer latencies when attaching to a nipple in the presence of the odor paired earlier with LiCl (Smotherman, 1982b). Experiments such as these have clearly demonstrated that rat fetuses can learn as early as Day 20 of gestation, yet these studies have been limited to postnatal assessment of learning. Little is yet known about the responses of fetuses in utero to unconditioned and conditioned stimuli.

A second experimental approach has overcome these limitations by allowing direct observation of fetal behavior. A pregnant female rat is prepared by surgically or chemically transecting the spinal cord, which produces permanent spinal anesthesia. The uterus is then externalized, the preparation is placed in a temperature-controlled saline bath, and the fetuses are allowed to float freely. This procedure enables the direct observation of spontaneous fetal behavior and has been applied to the study of behavioral development (e.g., Bekoff & Lau, 1980; Narayanan, Fox, & Hamburger, 1971; Smotherman, Richards, & Robinson, 1984) and the effect of drugs administered prenatally (Kirby & Holtzman, 1982; Narayanan, Narayanan, & Browne, 1982).

In the present study, this technique for directly observing fetal behavior was used

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to assess whether rat fetuses can learn taste/odor associations earlier than Day 20 of gestation. A series of experiments was conducted to answer three separate but related questions: (a) Will fetuses respond to a taste/odor cue, such as mint, as a function of its novelty or familiarity? (b) How do fetuses react to an aversive stimulus, such as LiCl, when presented at various gestational ages? (c) Can fetal behavior be conditioned and expressed in utero after the pairing of a taste/odor stimulus and LiCl?

General Method

Subjects

Adult female Sprague-Dawley rats (Simonsen Laboratories, Gilroy, California) were bred to Long-Evans males to produce fetuses used as subjects 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. Until Day 17 of gestation, females were housed in polycarbonate cages (32.7 × 37.8 × 9.5 cm) in groups of three per cage; on Day 17 they were rehoused and maintained in individual cages until the day of observation. Cages remained in a temperature- and humidity-controlled colony room with lights on from 1100 to 2300 daily. Food and water were available ad lib.

Day 17 Stimulus Exposure/Injection

On Day 17 of gestation, between 1200 and 1700, fetuses were exposed to a taste/odor stimulus by injection into the amniotic fluid (Experiment 1), injected ip with lithium chloride (Experiments 2A and 2B), or both (Experiments 3 and 4). The pregnant females were anesthetized with ether, a midline laparotomy was performed, and both horns of the uterus were externalized to manipulate the fetus nearest the ovary in each horn. The taste/odor stimulus (the conditioned stimulus; CS) was prepared by mixing 1.0 ml of pure mint extract (Schilling brand) in 3.0 ml of isotonic saline. This mixture was centrifuged, and the supernatant oil was drawn off. The remaining mint-in-saline solution was used as the taste/odor stimulus in Experiments 1, 3, and 4. Fetuses were exposed to this mint solution or to saline by injecting 0.02 ml into the amniotic fluid surrounding the fetus in the vicinity of its nose and mouth. Our observations of food dyes injected into the amniotic fluid of adjacent fetuses showed little or no mixing over a period of several days; this suggests that there is negligible exchange of injected substances among fetuses within a uterine horn, and there is no evidence of interamniotic exchange between fetuses in different uterine horns. In Experiments 2A, 2B, 3, and 4, a solution of 0.15 M LiCl (the unconditioned stimulus; US) or isotonic saline was administered to fetuses by inserting a 30-

ga. hypodermic needle through the wall of the uterus and injecting the fetus ip with 0.01 ml of the solution. Following exposure or injection, the uterine horns were washed with isotonic saline and replaced in the peritoneum of the pregnant female, and the incision was sutured closed. This procedure for presenting fetuses with CS and US follows closely the procedures described by Blass and Pedersen (1980) and Stickrod (1981). Throughout this study, no fetuses were seen to be adversely affected by amniotic or ip injections.

Surgical Preparation

In Experiments 2A and 3, fetuses were observed on Day 17 immediately after LiCl or saline injection; in all other experiments, pregnant females were surgically prepared for observation of fetuses on Day 19. Under ether anesthesia, each female underwent a chemomyelotomy. This surgical procedure, which involves injection of 100% ethyl alcohol into the spinal cord at the T12-L1 level, produces an irreversible spinal anesthesia posterior to the site of injection (Basmajian & Ranney, 1961; Narayanan et al., 1982). Chemomyelotomy was selected over an alternative procedure—spinal transection—for its simplicity, consistency between preparations, and comparability to procedures in other recent studies of fetal behavior (see Smotherman et al., 1984, for a detailed comparison of these two procedures). Following the chemomyelotomy, the female was restrained in a Plexiglas holding apparatus, the uterus was exteriorized through a midline laparotomy, and the female's uterus and lower body 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 recovered from ether anesthesia and acclimated to the water bath for 20 min before behavioral observations began. All observations of fetal behavior took place between 1200 and 1700.

Day 19 Stimulus Exposure/Injection

Following acclimatization to the bath, 1 of the 2 subject fetuses received a second exposure to the taste/odor stimulus by injection into the amniotic fluid (mint or saline; Experiments 1 and 4) or received an ip injection of LiCl or saline (Experiment 2B). Immediately after exposure/injection, the fetus in its amnion was delivered from the uterus, with care taken to preserve the integrity of the placental-uterine attachment, and the behavior of the fetus was observed through the amniotic membrane. The exposure/injection manipulation was then repeated for the second subject fetus in the other uterine horn, and a second period of observation was conducted. All fetuses in this study exhibited good condition throughout observations, and there was minimal separation of the placenta from the uterus.

Behavioral Observations

Each fetus was observed for 10 min following stimulus manipulation and delivery from the uterus. Two persons cooperated to observe and record fetal behav-

ior during an observation session. One person, who was blind to the treatment, watched the subject fetus, categorized spontaneous behavior, and verbally called out the behavioral events as they occurred. The second person translated the events into a written record and monitored the temperature of the water bath, elapsed time, and the condition of the mother. We have found measures of reliability with this scoring system to be consistently high. In order to provide a measure of elapsed time and the temporal patterning of behavior, each 10-min observation period was subdivided into 15-s intervals by the recorder.

Categories of Behavior

The methods described by Sotherman et al. (1984) were followed: Observed behavior was sorted into seven exclusive and exhaustive categories of spontaneous fetal behavior and one category of maternal activity. The seven categories of fetal behavior included movements of the head, mouth, foreleg, and hindleg, twitch (a spasm along the side of the trunk), curl (a flexion or torsion of the trunk), and stretch (an extension or straightening of the trunk). In addition, any movement of the restrained female during an observation period that caused the uterus and fetuses to be moved was recorded *mother active*.

Analysis of Data

In order to describe more inclusive patterns of behavior, four additional categories were derived from the seven basic categories of fetal behavior. Complex activity included all instances of activity that comprised two or more simultaneous fetal behaviors. Two measures of overall fetal activity were used. Total whole activity was the number of times a fetus was recorded as active (i.e., the number of bouts of activity) regardless of whether the activity involved single or complex movements. Total component activity was the sum of all behavioral movements of a fetus. Component activity differed from whole activity in the way complex actions were scored; each behavioral component in a simultaneous complex event (e.g., stretch, foreleg, and hindleg) was counted separately to arrive at component activity. Finally, periods of inactivity reflected the number of 15-s intervals in which a fetus showed no activity. Frequency counts in each of the seven basic categories of fetal behavior, and in the derived category of complex activity, were also converted to percentage scores (frequency/component activity) to reflect the way behavior was distributed among categories.

Experiment 1: Response to Novel and Familiar Taste/Odor Stimuli

This experiment was conducted to ascertain whether the taste/odor stimulus to be used in later conditioning tests (a mint solution) would elicit responses from fe-

tuses when presented alone. A nonneutral reaction to a taste/odor stimulus could occur when fetuses are exposed to either a novel stimulus or a stimulus made familiar through preexposure. The experiment was designed to assess three possible outcomes that would indicate that the mint exposure in itself was influencing fetal behavior. These three outcomes were (a) a differential response to the mint exposure at the time of observation on Day 19, (b) a residual effect from the initial mint exposure on Day 17, expressed at the time of observation on Day 19, and (c) a differential response to a mint exposure on Day 19 as a function of its novelty versus familiarity.

Method

A sample of 32 females was used in Experiment 1, which provided a total of 64 fetuses for observation. On Day 17, and again on Day 19 before observation, 2 subject fetuses in each female received injections in the amnion, one of mint and the other of saline, producing four experimental groups: saline-saline (SS), saline-mint (SM), mint-saline (MS), and mint-mint (MM). Frequency counts and percentage scores in each behavioral category were compared by three-way (Day 17 exposure by Day 19 exposure by order) analyses of variance (ANOVA).

Results and Discussion

None of the 11 categories of fetal behavior, or the activity of the mother, was affected by exposure to mint on Day 17, as evidenced by the lack of Day 17 main effects (Figure 1). Furthermore, the analysis revealed only a few patterns of behavior that were affected by exposure to mint on Day 19. A main effect of Day 19 exposure was apparent for frequencies of twitch, $F(1, 56) = 7.17, p < .01$, and complex activity $F(1, 56) = 5.96, p < .05$, with both patterns exhibiting higher rates of occurrence when fetuses were presented with mint. In addition, complex activity, when expressed as a percentage of total activity, differed significantly between mint and saline exposures, $F(1, 56) = 4.57, p < .05$. Complex activity constituted a higher proportion of total activity among fetuses exposed to mint on Day 19 than fetuses exposed to saline.

In contrast, 6 of the 12 patterns showed significant main effects of order, in every case with the second fetus observed exhib-

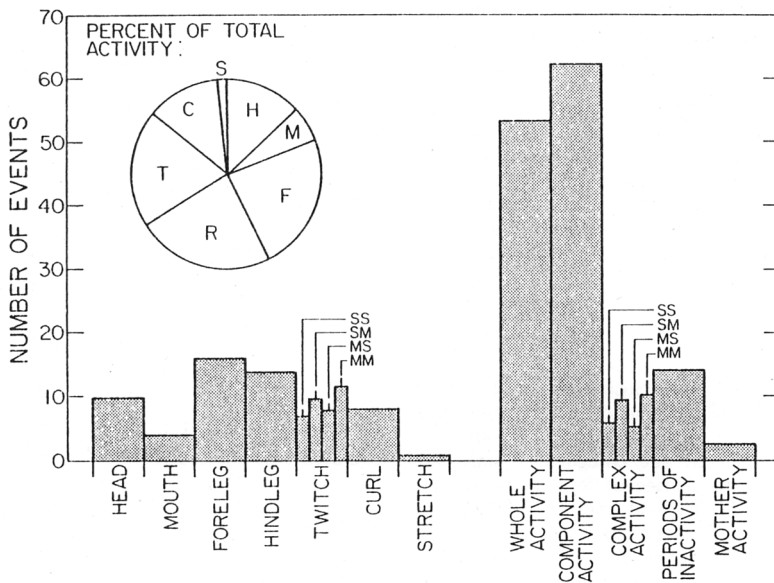


Figure 1. Number of behavioral events for 11 categories of fetal behavior and 1 category of maternal activity observed in Experiment 1. (Wide bars indicate mean frequencies when there were no significant differences between the four experimental conditions. Data for twitch and complex activity, for which significant differences were evident, are depicted as separate means for the four experimental groups: SS (saline on Day 17; saline on Day 19), SM (saline; mint), MS (mint; saline), and MM (mint; mint). Vertical lines represent SE. The sections within the circle graph represent the mean percentage of total activity for the seven basic patterns of fetal movement: H [head], M [mouth], F [foreleg], R [hindleg], T [twitch], C [curl], and S [stretch].)

iting higher rates of activity than the first. Main effects of order were found for frequency counts of whole activity, $F(1, 56) = 4.33, p < .05$, component activity, $F(1, 56) = 5.32, p < .05$, hindleg, $F(1, 56) = 4.13, p < .05$, head, $F(1, 56) = 4.70, p < .05$, curl, $F(1, 56) = 3.95, p < .05$, and complex activity, $F(1, 56) = 5.30, p < .05$. When data were converted to percentage of total activity, main effects of order were also apparent for twitch, $F(1, 56) = 4.37, p < .05$, and mouth movements, $F(1, 56) = 3.87, p < .05$, with the first fetus observed devoting a higher proportion of overall activity to twitch and mouth movements than the second fetus observed. No significant interactions among Day 17 exposure, Day 19 exposure, and order were observed for any category of behavior, whether expressed as frequency counts or percentage of total activity. The presence of significant order effects and the absence of Order \times Treatment interactions are consistent with our earlier research on fetal behavior (Smotherman et al., 1984).

The results of this experiment allow us to evaluate the three hypothetical outcomes of mint exposure outlined earlier. (a) Only two patterns of behavior—twitch and complex activity—were affected by immediate mint exposure on Day 19. In both cases, rates of activity were higher among fetuses exposed to mint immediately prior to observation (Groups SM and MM) than fetuses exposed to saline (Groups SS and MS). (b) Fetuses exposed to mint on Day 17 (Groups MS and MM) showed no difference among patterns of behavior when compared with fetuses exposed to saline on Day 17 (Groups SS and SM). Therefore, there is no evidence that the mint exposure on Day 17 has any residual effect on the behavior of fetuses on Day 19. (c) The fact that no interaction was apparent between Day 17 and Day 19 exposures argues that the influence of mint does not change as a function of its repeated presentation. Mint does not differentially affect fetal behavior when presented on Day 19 either as a novel stimulus (Group SM) or as a familiar stim-

ulus (Group MM). Overall, mint presentation in itself does not appear to influence fetal behavior greatly, and it does so only through a selective activation of individual behavioral patterns.

**Experiment 2:
Responses to an Aversive Stimulus,
Lithium Chloride**

There is some evidence that rat fetuses will respond to LiCl as an aversive stimulus. Stickrod et al. (1982a) reported their casual observation that fetuses on Day 20 of gestation reacted to LiCl injection by "wriggling and contracting." Experiments 2A and 2B were conducted to quantitatively characterize the response of fetuses to an unconditioned aversive stimulus—LiCl—at two gestational ages, Days 17 and 19.

Method

Eight females were prepared in each of the studies of Experiment 2, for a total of 16 subject fetuses in each study. Within a mother, both subject fetuses received the same ip injection, either LiCl or saline, on either Day 17 (2A) or Day 19 (2B). Fetuses were observed immediately after the injection. In both studies, the data were subjected to a two-way (Injection × Order) ANOVA.

Results and Discussion

Experiment 2A: Day 17 injection and observation. Two categories of fetal behavior were affected by LiCl injection on Day 17 (Figure 2). Foreleg movements were less frequent in LiCl-treated fetuses than in saline-treated controls, as indicated by a significant main effect of injection, $F(1, 28) = 6.96, p < .01$. Foreleg movements were also affected by LiCl when viewed as a percentage of total activity, $F(1, 28) = 9.95, p < .01$, constituting a smaller proportion of overall activity among LiCl-treated fetuses than saline-treated. The pattern of response was reversed for curls; when expressed as a percentage, the main effect of injection was significant, $F(1, 28) = 11.16, p < .01$, a result indicating that curls formed a higher proportion of total activity among LiCl-treated fetuses. No order main effects were observed for frequency counts of behavioral patterns, and only one pattern showed a significant effect when analyzed as percentage of total activity. Foreleg movements were more frequent during the second 10-min period of observation, $F(1, 28) = 4.76, p < .05$.

The results of this experiment show that few patterns of fetal behavior are affected

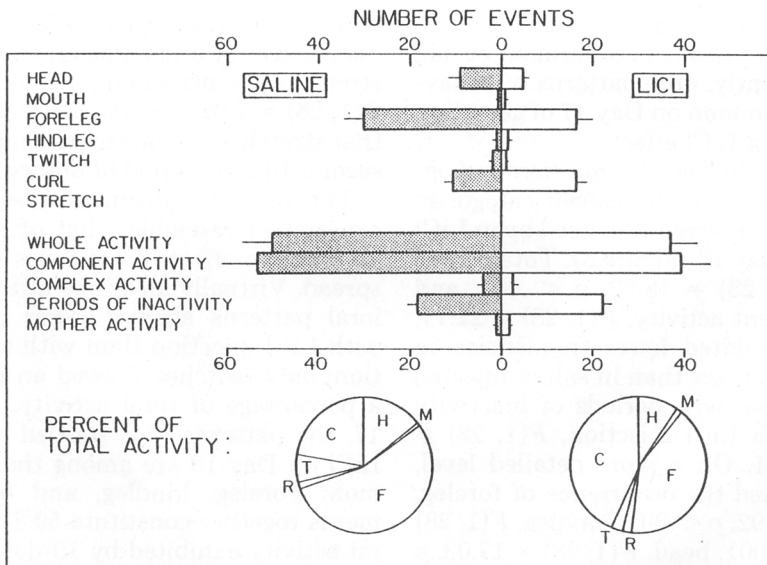


Figure 2. Number of behavioral events observed in Experiment 2A for fetuses given saline or LiCl and observed on Day 17 of gestation. (Bars represent mean frequencies; horizontal lines show SE. Sections of each circle show the percentage of total activity for the seven basic movement patterns of fetuses given saline or LiCl. For meaning of abbreviations, see caption to Figure 1.)

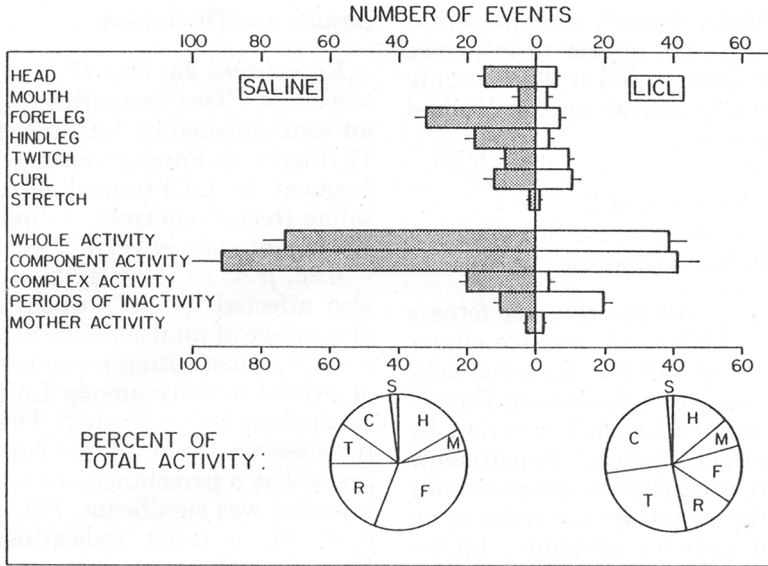


Figure 3. Number of behavioral events observed in Experiment 2B for fetuses given saline or LiCl and observed on Day 19. (Bars represent mean frequencies; horizontal lines show SE. Sections of each circle show the percentage of total activity for the seven basic movement patterns of fetuses given saline or LiCl. For meaning of abbreviations, see caption to Figure 1.)

by LiCl injection on Day 17. The two categories of behavior that were affected by LiCl showed different patterns of change: Foreleg movements were suppressed, and curls increased in frequency. These two behavioral patterns together represent 63.4% of the total activity shown by 17-day-old rat fetuses (Smotherman et al., 1984). Apparently, only patterns of behavior that are common on Day 17 of gestation are subject to a LiCl effect.

Experiment 2B: Day 19 injection and observation. Eight of the eleven categories of fetal behavior were influenced by ip LiCl injection on Day 19 (Figure 3). Total whole activity, $F(1, 28) = 18.22, p < .001$, and total component activity, $F(1, 28) = 22.77, p < .001$, exhibited lower frequencies in LiCl-treated fetuses than in saline-injected controls. Conversely, periods of inactivity increased with LiCl injection, $F(1, 28) = 11.21, p < .01$. On a more detailed level, LiCl suppressed the occurrence of foreleg, $F(1, 28) = 41.92, p < .001$, hindleg, $F(1, 28) = 22.06, p < .001$, head, $F(1, 28) = 17.03, p < .001$, and complex, $F(1, 28) = 33.65, p < .001$, movements. Main effects of injection were also evident among three behavioral categories when data were expressed as a

percentage of total activity. Twitches constituted a higher proportion of all activity with LiCl injection, $F(1, 28) = 14.09, p < .001$, and foreleg, $F(1, 28) = 39.21, p < .001$, and complex activity, $F(1, 28) = 51.56, p < .001$, were markedly suppressed by LiCl relative to other patterns of behavior. A main effect of order was apparent only for stretch as a percentage of total activity, $F(1, 28) = 7.02, p < .05$, a result indicating that stretches became more common in the second 10-min period of observation.

The overall pattern of results in this experiment resembles that of Experiment 2A, but the effect of LiCl was more widespread. Virtually all of the affected behavioral patterns showed fewer occurrences with LiCl injection than with saline injection; only twitches showed an increase, as a percentage of total activity. As on Day 17, the patterns that showed an effect of LiCl on Day 19 are among the most common: Foreleg, hindleg, and head movements together constitute 59.8% of the total activity exhibited by 19-day-old rat fetuses, and complex activity comprises 15.2% of all component acts (compared with 6.8% on Day 17). No effects of order were evident, a finding that differs from

those in Experiment 1 and in earlier studies of fetal behavior (Smotherman et al., 1984). The results of Experiments 2A and 2B thus argue that LiCl injection affects fetal behavior at both ages, but as developmental changes in behavior occur, more patterns of behavior become susceptible to suppression by LiCl. In addition, the evidence of increased curl and twitch movements suggests that LiCl has certain specific and independent effects on individual behavioral patterns.

Experiment 3: Response to a Taste/Odor Stimulus Paired With LiCl

Experiment 1 demonstrated that mint presentation had little effect on fetal behavior in utero; only two categories of behavior were affected, twitch and complex activity, which showed a slight increase. Experiments 2A and 2B revealed a very different pattern of response to ip LiCl injection; overall activity was greatly reduced, and virtually all specific categories of movement became less frequent. Because mint exposure and LiCl injection have very different and nearly opposite effects on fetal behavior, it is impossible to know a priori what effect the pairing of mint with LiCl will have on fetuses. The purpose of Experiment 3 was to ascertain how fetuses respond to amniotic mint presentation followed by ip LiCl injection on Day 17.

Method

Twelve females provided subject fetuses for treatment and observation in Experiment 3. On Day 17, each subject fetus was exposed to either mint or saline through injection into the amnion, followed within 60 s by ip injection of LiCl. Immediately after the LiCl injection, each subject fetus was delivered from the uterus into the bath and observed. Because each female provided one saline and one mint subject fetus, the data were compared with *t* tests for matched pairs.

Results and Discussion

Fetuses in both mint and saline groups exhibited low rates of activity, comparable to the levels of activity of LiCl-treated fetuses in Experiment 2A (e.g., mean total component activity: saline = 30.3 ± 8.3 ; mint = 34.9 ± 5.0 ; in Experiment 2A, 39.9

± 6.3). Twitch and complex activity, which were activated by mint presentation in Experiment 1, showed no indication of increased incidence in the mint group of Experiment 3. In fact, none of the 11 patterns of fetal behavior were affected differently by the mint and saline exposures, all $t(11) < 1.25$, $ps < .10$. These results suggest that fetuses responded exclusively to the ip LiCl injection and were not influenced by the presence of mint.

Experiment 4: Response to a Taste/Odor Stimulus Conditioned Through Pairing With LiCl

Once the responsiveness of fetuses to a taste/odor stimulus such as mint (Experiment 1), to an unconditioned aversive stimulus such as LiCl (Experiment 2), and to the pairing of mint and LiCl (Experiment 3) is known, it is possible to test for the expression of a learned aversion in utero. Experiment 4 addressed three questions: (a) Can a fetus learn at an earlier age than has yet been reported? (b) Will a fetus alter its motor activity in response to a conditioned stimulus and thus demonstrate expression of a learned aversion in utero? (c) Does the conditioned response of a fetus during performance of a learned aversion resemble the unconditioned fetal response to an aversive stimulus, LiCl?

Method

Twelve females provided subject fetuses for treatment and observation in Experiment 4. On Day 17, each subject fetus was exposed to either mint or saline through injection into the amnion, followed by ip injection of LiCl. On Day 19, after the prepared female was placed in the water bath, each subject fetus was delivered from the uterus, exposed to mint, and observed. Data from the two experimental groups (saline-mint and mint-mint) were compared with *t* tests for matched pairs.

Results and Discussion

Six of the eleven patterns of fetal behavior were affected differently by the mint-mint and saline-mint exposures (Figure 4). Total whole activity, $t(11) = 2.58$, $p < .05$, total component activity, $t(11) = 2.90$, $p < .02$, and complex activity, $t(11) = 2.36$, $p < .05$.

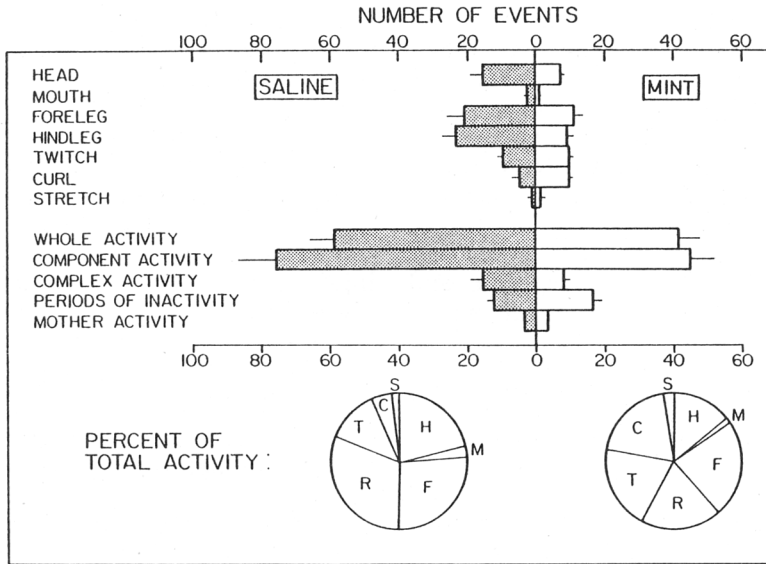


Figure 4. Number of events of fetal behavior observed in Experiment 4. (Fetuses were exposed to either saline or mint, then given LiCl on Day 17, and were reexposed to mint before observation on Day 19. Bars represent mean frequencies; horizontal lines show SE. Sections of each circle show the percentage of total activity for the seven basic movement patterns of fetuses exposed to saline or mint. For meaning of abbreviations, see caption to Figure 1.)

.05, differed significantly between mint-mint and saline-mint fetuses. Different responses were also evident for individual patterns of behavior, including hindleg, $t(11) = 4.28, p < .005$, curl, $t(11) = 2.92, p < .02$, and head, $t(11) = 2.43, p < .05$, movements. All of these patterns of general activity and specific movement except curl and periods of inactivity showed lower frequencies in the mint-mint group than the saline-mint group; curls and inactivity, however, reversed this pattern with an increase in frequency in the mint-mint condition. When data were analyzed as a percentage of total activity, two behavioral categories, curl and twitch, exhibited a differential response. Curls showed a large change, constituting 22.3% of all activity in the mint-mint group, compared with only 7.4% in the saline-mint group, $t(11) = 4.13, p < .05$. Twitches also showed an increase in relative occurrence, from 14.1% in saline-mint group to 22.8% in mint-mint group, $t(11) = 2.51, p < .05$. Among the other patterns of fetal behavior, most of them showed small decreases in percentage of activity in the mint-mint condition, although none of these differences were statistically significant.

Overall, the results of Experiment 4 demonstrate a clear response to the mint solution on Day 19 as a function of its pairing with LiCl injection on Day 17. Because both saline-mint and mint-mint fetuses were injected with LiCl on Day 17, the behavioral differences cannot be ascribed to a long-term effect of LiCl. Similarly, because both groups received mint on Day 19, the behavioral differences cannot be due to any immediate effect of the mint stimulus. Furthermore, the findings of Experiment 1 indicate that the Day 17 mint exposure does not produce a residual effect or a familiarity effect on Day 19 responsiveness, and those of Experiment 3 show no effect of mint on fetal responsiveness to LiCl injection on Day 17 which might persist to Day 19. Thus, the results of Experiment 4 provide evidence that (a) rat fetuses are capable of forming learned associations between neutral and aversive stimuli as early as Day 17 of gestation and (b) the behavior of fetuses in utero can be influenced by associations formed at an earlier gestational age.

The pattern of response to the mint CS closely resembles the response to the LiCl-US as it appears on Day 19 (Experiment

2B). Total whole activity, total component activity, complex activity, and hindleg and head movements were all suppressed by both the LiCl US and the mint CS, and twitch increased as a percentage of total activity with both LiCl and mint. Foreleg movements decreased significantly with LiCl injection on Day 19, and showed a significant decrease with the mint CS. Conversely, periods of inactivity showed a significant increase with the mint CS. Only for curl is a clear difference between CS and US apparent: Curls increased sharply in frequency in response to the mint CS but showed no increase when LiCl was injected on Day 19. Curl movements also showed an increase in frequency after LiCl injection on Day 17 (Experiment 2A), which suggests that fetuses respond to a taste/odor CS by reexpressing patterns of behavior that were common at the time the aversion was formed.

General Discussion

The findings of these experiments provide answers to the three questions posed at the beginning of this article. Experiment 1 shows that rat fetuses exhibit little change in behavior to the taste/odor of mint presented in utero. Only two patterns of behavior—twitch and complex activity—are affected by mint presentation on Day 19. There is no evidence of long-lasting effects of exposure to mint, or of any influence of familiarity to the mint stimulus. Moreover, Experiment 3 demonstrates that the presence of mint does not alter fetal responsiveness to the aversive stimulus, LiCl. Therefore, the taste/odor of mint is neutral, relative to isotonic saline, when presented to fetuses, and in the few cases in which it does influence fetal behavior, it serves to activate or increase the frequency of movement.

Experiments 2A and 2B reveal that rat fetuses respond to ip LiCl injection with a suppression of overall activity. Fetuses exhibit a smaller repertoire of behavior on Day 17 than on Day 19, and, not surprising, fewer patterns of behavior are affected by LiCl on Day 17 than on Day 19. On both days, however, nearly all individual categories of behavior shown by fetuses were affected. Not all patterns are suppressed

uniformly by LiCl, as indicated by significant changes in the percentage of total activity for foreleg and curl on Day 17, and twitch, foreleg, and complex activity on Day 19. Moreover, curl movements actually increase on Day 17 after LiCl injection. This unequivocal pattern of response is evidence that LiCl does not merely debilitate fetuses and thereby suppress all behavior. Rather, LiCl alters the way fetal behavior is distributed among various behavioral categories, selectively suppressing some actions while activating others. Judging from the different response of curl movements on Day 17 and Day 19, the effect of LiCl on the behavioral repertoire also changes slightly with gestational age. Apparently, the aversive consequences of LiCl injection differ in 17-day-old and 19-day-old rat fetuses.

Experiment 4 clearly demonstrates that rat fetuses can acquire a conditioned aversion to mint on Day 17 of gestation. This is the earliest age yet found at which conditioning can be effected, 3 days earlier than in previous reports on fetal conditioning (Smotherman, 1982a, 1982b; Stickrod et al., 1982a, 1982b). Experiment 4 also shows that the conditioned aversion can be expressed in utero through its effects on spontaneous fetal behavior. Fetal activity is suppressed after presentation of the mint CS, a pattern of response that resembles the behavior shown immediately after an injection of LiCl.

One of the reasons for conducting Experiments 2A and 2B was to provide a basis for comparison with Experiment 4. In particular, fetuses might show a pattern of response to the mint CS that is equivalent to what they would have shown to LiCl had they been injected on Day 19, or they might show a regression (in some sense) to the patterns of behavior they showed when LiCl was injected on Day 17. Evidence from Experiment 4 is mixed. The responses of fetuses to the mint conditioned stimulus are very similar to their responses to LiCl on Day 19; of the 11 categories of fetal behavior, 10 show the same trend in Experiments 2B and 4. The one notable exception is curl, which increased in frequency in response to the mint CS but showed a slight decline after the injection of LiCl on

Day 19. Only on Day 17 was this pattern of increase for curl evident after the LiCl injection. This finding is consistent with the idea that fetuses are reexpressing behavior that they showed at the time the conditioned aversion was formed.

In conclusion, this study demonstrates that chemical modifications of the uterine environment can affect fetal behavior. Although it evaluates only the effects of experimental manipulations of the uterine environment, this study may have larger implications for possible effects on fetal behavior during normal pregnancy. Many chemical substances are known to cross the placental barrier intact. Some of these chemicals, such as alcohol (McLeod et al., 1983) and other drugs (Kirby, 1981), produce a suppression of behavior similar to the aversive effects of LiCl. Taken together, these facts satisfy the necessary conditions for establishing taste/odor aversions in utero. Natural associations formed in utero may influence postnatal maternal-offspring behavior (DeCasper & Fifer, 1980; Pedersen & Blass, 1982), dietary preferences (cf. Galef & Sherry, 1973), preferences for novel or familiar tastes and odors (Smotherman, 1982a), and other forms of behavior. Similarly, chronic exposure to behavior-suppressing substances may retard the development of coordination and complex behavior (Hofer, 1981, p. 189) in a manner analogous to the way early postnatal deprivation of sensory experience profoundly affects later perceptual capability (e.g., Hirsch & Spinelli, 1971). It remains for future research to investigate the true role of spontaneous fetal behavior and the potential for fetal learning in subsequent behavioral development.

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