

## Habituation in the Rat Fetus

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Rat fetuses exhibit motor and cardiac responses to chemosensory stimulation on Days 20 and 21 of gestation. The first experiment demonstrated that fetuses exhibit an increase in overall motor activity and decrease in heartrate in response to an initial intraoral infusion of a lemon solution. After a series of nine exposures, however, fetuses no longer exhibit motor or cardiac responses to lemon infusion, suggesting the existence of a habituation-like process. Responsiveness recovers spontaneously following a 3- to 9-min period without stimulation. In a second experiment, a dishabituation treatment was administered to distinguish habituation, which is a centrally mediated decrement in response, from effector fatigue, sensory adaptation, and other peripheral mechanisms that can result in reduced responsiveness. A single infusion of mint following a series of nine lemon exposures was effective in reinstating fetal motor responses to lemon on both Days 20 and 21, but reinstated cardiac responses only on Day 21. Rat fetuses habituate to repeated chemosensory stimulation, suggesting the utility of the habituation paradigm in measuring CNS development during the perinatal period.

A common theme in developmental research is the importance of early events in shaping the course of ontogeny. Although this general statement is true for physical growth and development, research conducted over the past three decades has repeatedly confirmed the crucial role of early sensory experience in behavioural and cognitive development (Bell & Smotherman, 1980). Research interest in prenatal development has provided information concerning the role of sensory experience in behavioural development before birth (Smotherman & Robinson, 1987; Robinson &

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Smotherman, 1991). Because all experience is founded on an animal's ability to sense changes in its environment, measurement of the fetus's sensory capacities defines the limits of its potential for modifying its behaviour through experience.

Fetuses develop within a complex environment, the uterus, that permits access to sensory stimuli in many modalities (Smotherman & Robinson, 1988a). Furthermore, different sensory systems exhibit function before birth. In particular chemosensory systems, such as olfaction, exhibit considerable plasticity in both morphological development and involvement in lasting behavioural changes (Leon, 1987; Leon et al., 1987). It is certain that chemosensation plays a crucial adaptive role in regulating maternal-infant interactions and infant feeding immediately after birth (Blass, 1986; Hudson, 1985; Macfarlane, 1975; Vince & Ward, 1985). It follows that chemically directed behaviour, and the capacity to modify behavioural responses with chemosensory experience, must be capable of expression before birth.

Habituation is a process whereby organisms can modify their behaviour through sensory experience. Habituation has generally been viewed as a simple form of learning shared by virtually all animals that possess a central nervous system (Carew & Sahley, 1986). The reason for the primacy of habituation is fundamental: organisms must ignore frequently encountered stimuli that bear little or no consequence if they are to maintain an ability to recognize and respond to novel and important stimulus features in their environment. Habituation encompasses "all response decrements (a) associated with repetitious presentations of a stimulus, where (b) the stimulus is not a conditioned stimulus, and (c) where sensory adaptation, fatigue, satiation, and inherent rhythms may be discounted as causal factors" (Kling & Riggs, 1971, p. 588).

Measurement of response decrement to repeated stimulation has become a standard technique for assessing the state of central nervous system development in human infants (Jeffrey & Cohen, 1971), particularly in pre-term and high-risk infants (Lester & Tronick, 1990). Attempts to study habituation in human fetuses through the application of vibroacoustic stimuli to the maternal abdomen have also been reported (Leader, Baillie, Martin, & Vermeulen, 1982a, 1982b; Lecanuet, Granier-Deferre, Cohen, LeHouezec, & Busnel, 1986). The need to conduct critical experiments that distinguish a central process such as habituation from peripheral causes of response decrement has been recognized only recently in studies of prenatal development (Madison et al., 1986).

In order to demonstrate habituation unambiguously, experiments must be conducted to exclude receptor adaptation, effector fatigue, and inherent rhythms as possible factors underlying decreased responsiveness to stimulation. A broadly accepted experimental approach is (1) to determine the

latency necessary to permit spontaneous recovery of the response, (2) to present a less intense or qualitatively different stimulus within this latency to effect dishabituation, and (3) to demonstrate increased responsiveness to the original stimulus following dishabituation. In the present study, these criteria are applied to examine habituation to repeated chemosensory stimulation in the rat fetus.

## GENERAL METHODS

*Subjects.* Fetuses used as experimental subjects in this study were the progeny of time-mated Sprague-Dawley rats (Charles River Laboratories, Wilmington, Massachusetts). Pregnant females remained housed in groups of three in polycarbonate cages (38 × 50 × 20 cm) until testing on Day 20 or 21 of gestation (presence of sperm in vaginal smear = Day 0). Cages were kept in a temperature- and humidity-controlled colony room; lights were programmed to follow a 12:12 hr light:dark cycle (on at 0700). Rats were provided with ad libitum food and water and maintained in accordance with guidelines for animal care established by the National Institutes of Health (PHS publication no. 86-23).

*Maternal Preparation.* To provide access to the fetus late during gestation, pregnant rats were placed under brief ether anaesthesia and prepared by chemomyelotomy, a procedure that results in irreversible chemical transection of the spinal cord (Smotherman, Richards, & Robinson, 1984). Chemomyelotomy was performed by injecting 100  $\mu$ l of 100% ethanol into the spinal canal between the first and second lumbar vertebrae. Rats prepared by this method lack sensation on the posterior half of the body, permitting surgical procedures without general anaesthesia, which suppresses fetal motor activity. The prepared female was placed in a plexiglas holding apparatus, the uterus externalized through a low midline incision, and the uterus and lower body immersed in a bath containing a buffered saline solution maintained at 37.5°C. A minimum of 20 min elapsed before the beginning of experimental manipulation or observation of fetuses to provide ample time for the mother and fetuses to recover from the ether anaesthetic and to acclimatize to the bath.

*Fetal Preparation.* Subject fetuses were externalized from the uterus prior to behavioural testing. Each subject was delivered through a small incision in the uterine wall, removed from its enveloping embryonic membranes, and freely suspended within the saline bath. Particular care was taken to ensure the integrity of the fetus's umbilical connection to the placenta and uterus; all fetuses used as subjects remained healthy (as indicated by pink colouration) throughout the session of observation and testing.

To enable chemosensory stimulus delivery, an intraoral cannula, consisting of a 2–3 cm length of PE-10 polyethylene tubing, was implanted through the lower jaw of the subject with a flanged tip resting on the dorsal surface of the tongue in a mid-anterior position (Kehoe & Blass, 1985). In Experiment 2, which called for the sequential delivery of two different solutions, a dual cannula with two independent channels was implanted. The free end of the cannula was friction-fitted to a length of PE-50 tubing, which in turn was connected to an automated syringe pump (Kashinsky, Rozboril, Robinson, & Smotherman, 1990) containing the test solution. This apparatus permits delivery of fluid stimuli into the oral cavity of the fetus with precise control over the volume and timing of infusion. Previous reports have documented that the procedures employed to install the cannula have no influence on the amount or pattern of spontaneous fetal activity (Smotherman & Robinson, 1988b, 1991). In the present study, two different chemosensory fluids with strong olfactory characteristics—lemon and mint—were used as test stimuli (Smotherman & Robinson, 1990a). These solutions were prepared as well-mixed 1:2 dilutions of commercially available extract (Schilling brand) in an isotonic saline carrier. Isotonic saline alone (0.9% NaCl) was used as an infusion control.

*Behavioural Testing.* Individual fetal subjects were tested at one of two ages, Day 20 or 21 of gestation (term = 21.5 days). Each subject fetus was observed during a test session that consisted of a 60-sec baseline period, a series of nine exposure trials, and a post-exposure period. Each exposure trial consisted of a 20  $\mu$ l infusion of the lemon solution delivered in a 1–2-sec pulse; trials were separated by an interval of 15 sec. The volume and concentration of the lemon solution and the temporal schedule of exposure trials were selected following a series of preliminary experiments that systematically varied these stimulus parameters. These preliminary experiments demonstrated that behavioural and cardiac responses ceased to be expressed by fetuses after a series of infusions under a variety of combinations of these parameters. The specific parameters used in the present experiments were selected because they consistently produced waning responsiveness in fetal subjects tested on Days 20 and 21 of gestation.

Throughout the test session, all movements of the subject fetus were noted by an observer and keyed into a microcomputer equipped with software to serve as a real-time event recorder. Counts of movement events, including movements of the head, mouth, forelimbs, rear limbs, and trunk, were totalled to provide a measure of overall fetal activity. This protocol has been employed in previous studies of fetal behaviour and found to be highly reliable in preserving a continuous record of fetal movements in correct temporal and sequential relation to the moment of stimulus delivery (Smotherman & Robinson, 1985, 1991). The index of

overall fetal activity was used to measure changes in fetal motor responses to repeated stimulation.

*Heart rate Recording.* Changes in heart rate (HR) were measured to provide an independent physiological measure of fetal responsiveness. Each subject fetus was fitted with paired cardiac leads fashioned from #36 nickel-chrome wire. The terminal insulation was stripped from the tips of the two leads, which were bent to a sharp angle and inserted subcutaneously over the sternum and mid-thoracic vertebrae. The leads were connected to a Grass model 79 polygraph, which amplified the EKG signal and printed a continuous paper record of heart beats. Fetal HR was measured from these strip records in 5-sec intervals and converted to beats per minute (bpm) for analysis (Smotherman & Robinson, 1988c).

*Data Analysis.* The 30-sec period following an infusion was divided in six 5-sec intervals to permit analysis of motor activity and cardiac responsiveness to stimulation. Because baseline motor activity remained stable across ages, the frequency of movement events in each interval was analysed directly. However, because fetal resting HR exhibited a significant increase from Day 20 to Day 21, it was necessary to convert absolute HR measurements to change scores to permit comparison across ages. Change scores were calculated as absolute HR during an interval subtracted from baseline HR during the 5 sec interval immediately preceding infusion (Smotherman & Robinson, 1988c). Motor activity and HR measures were analysed in multifactor ANOVAs to assess whether time-dependent changes in fetal response occurred as a function of gestational age or experimental treatment. In all ANOVAs, the 5-sec intervals factor was treated as a repeated measure. In addition to these analyses, correlation coefficients were calculated to determine whether the magnitude of cardiac response was influenced by the baseline HR of each subject (Richardson, Seigal, & Campbell, 1988).

## EXPERIMENT 1 WANING AND RECOVERY OF FETAL RESPONSE

The aim of this experiment was to characterize the decrement in fetal response to a series of lemon infusions and determine whether re-exposure to lemon after a period without stimulation would result in spontaneous recovery in responsiveness.

### Method

At each age, 20 fetuses (from 10 mothers) were exposed to the series of exposure trials consisting of nine 20  $\mu$ l lemon infusions delivered at 15-sec intervals. Following the ninth infusion, half of the subjects were retested

after a delay of 3 min and half after a delay of 9 min. The retest consisted of a single 20- $\mu$ l infusion of lemon. To assess waning and recovery in fetal responses, behavioural and cardiac data were collected immediately before and for 30 sec following the first exposure trial, the ninth exposure trial, and the retest infusion.

## Results and Discussion

Baseline levels of fetal motor activity and HR were measured at four points during the observation session: (1) prior to the first exposure trial, (2) prior to the ninth exposure trial, (3) prior to the retest infusion, and (4) at the conclusion of the observation session, 60 sec after the retest. Behavioural and cardiac measures were analysed in separate 2 (age)  $\times$  4 (baseline periods) ANOVAs (see Table 1). The baseline periods factor was treated as a repeated measure in these analyses. While fetal motor activity ranged from 0.9 to 1.8 movements per 5-sec interval, the ANOVA revealed no significant effects of age or baseline period. Fetal HR exhibited low variability within ages but differed significantly as a function of age,  $F(1, 38) = 12.8$ ,  $p < 0.001$ . HR was significantly higher on Day 21 than on Day 20. To compensate for age-related changes in resting HR, subsequent analyses of cardiac responses were conducted with HR change scores.

To assess changes in fetal responsiveness to the repeated presentation of the chemosensory stimulus, motor activity and HR change scores for the first and ninth exposure trials were compared in separate 2 (age)  $\times$  2 (trials)  $\times$  6 (5-sec intervals) ANOVAs. The analysis of motor activity indicated the significant Trials  $\times$  5-sec Intervals interaction,  $F(5, 190) = 8.4$ ,  $p < 0.001$ . At both ages, the first exposure trial resulted in a prompt increase in motor activity, which returned to baseline within

TABLE 1  
Baseline Motor Activity and HR in Experiment 1

Baseline Period	1	2	3	4
<i>Day 20</i>				
Activity (per 5 sec)	1.3	1.7	1.5	1.4
SEM	0.4	0.4	0.3	0.4
HR (bpm)	309.0	307.8	309.6	309.0
SEM	4.3	4.8	3.6	3.9
<i>Day 21</i>				
Activity	1.0	1.8	1.2	0.9
SEM	0.2	0.4	0.3	0.2
HR	328.2	326.4	324.0	324.0
SEM	5.2	5.9	4.3	4.3

30 sec. No change in motor activity was apparent during the ninth exposure trial (see Figure 1).

Cardiac responses to infusion were also evident during the exposure trials, as indicated by the significant Trials  $\times$  5-sec Intervals interaction,  $F(5, 190) = 2.8, p < 0.05$ . At both stages, a pronounced bradycardia occurred immediately after infusion in the first exposure trial, which persisted through the 30-sec period of measurement. Bradycardia was not evident, however, at any point during the ninth exposure trial (see Figure 1). The Pearson correlation indicated no relationship between the absolute HR during the baseline period and the magnitude of maximum bradycardia during the first exposure trial [Day 20:  $r(20) = -0.047$ , n.s.; Day 21:  $r(20) = -0.083$ , n.s.].

The motor and cardiac responses of fetuses to the retest infusion, which was presented 3 min or 9 min after the last exposure trial, were analysed in separate 2 (age)  $\times$  2 (delay)  $\times$  6 (5-sec intervals) ANOVAs. The analysis of motor activity indicated the significant main effect of delay,  $F(1, 36) = 6.4, p < 0.05$ . Fetuses retested after a 9-min delay were more active than fetuses retested only 3 min after exposure (see Figure 2).

Analysis of HR change scores in response to the retest infusion indicated the significant Age  $\times$  Delay  $\times$  5-sec Intervals interaction,  $F(5, 180) = 2.3$ ,

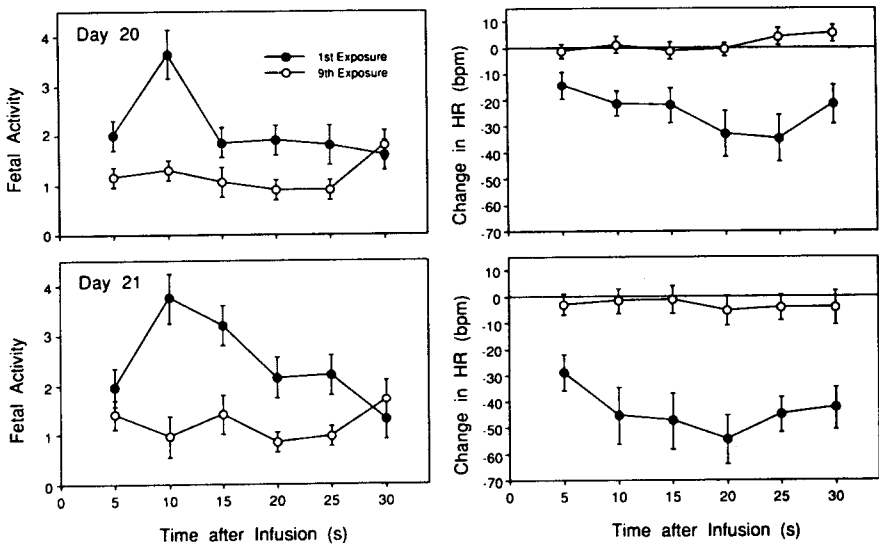


FIG. 1. Fetal motor activity (left panels) and change in HR (right panels) on Day 20 (top panels) and Day 21 (bottom panels) of gestation in response to lemon exposure in Experiment 1. Motor activity is expressed as the number of fetal movements per 5-sec interval. The fetal responses to the first lemon exposure and the ninth in a series of exposures are displayed. Points represent means; vertical lines depict SEM.

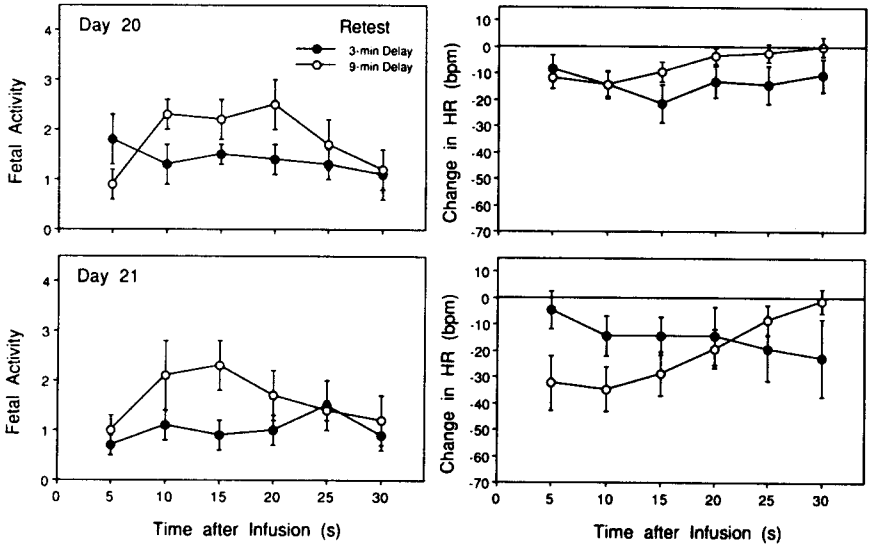


FIG. 2. Fetal motor activity and change in HR on Days 20 and 21 of gestation in response to the retest infusion of lemon in Experiment 1. Fetal responses when the retest is presented after a delay of 3 min or 9 min are displayed.

$p < 0.05$ . Tests for simple interaction effects were conducted separately for each age. On Day 20, HR did not vary significantly as a function of either 5-sec interval or period of delay. On Day 21, the interaction of Delay  $\times$  Intervals was significant,  $p < 0.01$ . The recovery in HR response was most evident among fetuses tested on Day 21 after a 9-min delay (see Figure 2). Pearson correlations showed no relationship between the absolute HR during the baseline period preceding the retest and the magnitude of bradycardia following infusion [Day 20:  $r(20) = -0.063$ , n.s.; Day 21:  $r(20) = -0.252$ , n.s.].

Considered together, the activity and HR data from the first experiment indicate that fetuses on Days 20 and 21 of gestation exhibit a distinctive response to their first exposure to a chemosensory stimulus. This initial response consists of an immediate increase in motor activity and simultaneous decrease in HR. A significant decrement in fetal response to the lemon stimulus occurs over a series of nine stimulus presentations, resulting in the absence of fetal motor and cardiac responses to infusion after the ninth lemon exposure. The retest results suggest spontaneous recovery of the motor response occurs when fetuses are retested 9 min after the last exposure trial. Furthermore, there is some evidence for recovery of the cardiac response to infusion, especially for fetuses tested on Day 21 after a delay of 9 min. There was no evidence that baseline HR was predictive of fetal cardiac response to stimulation. This pattern of initial responsiveness, wan-

ing response over a series of exposures, and recovery after a delay is consistent with the existence of a habituation-like process in the fetus. It is important to recognize, however, that these effects may be explained by peripheral effects such as adaptation of sensory receptors or motor fatigue.

## EXPERIMENT 2 HABITUATION AND DISHABITUATION

The aim of Experiment 2 was to employ a dishabituation procedure to reinstate fetal responsiveness to the chemosensory stimulus after its repeated presentation (Groves & Thompson, 1970; Jeffrey & Cohen, 1971; Madison et al., 1986).

### Method

In this experiment, 45 fetuses (from 15 mothers) were fitted with a dual cannula to permit sequential infusion of two different chemosensory solutions. All fetal subjects were exposed to a series of nine exposure trials involving lemon infusion following the same procedures used in Experiment 1. Sixty seconds after the ninth exposure trial, fetuses were exposed to one of three dishabituation treatments ( $n=15$  per treatment), involving no treatment (NT controls), 20  $\mu\text{l}$  infusion of isotonic saline (Saline controls), or 20  $\mu\text{l}$  infusion of a mint solution (Mint group). A final retest, consisting of a single 20- $\mu\text{l}$  infusion of lemon, occurred 60 sec after the dishabituation treatment. The total delay between the ninth exposure trial and the retest thus was 2 min, a period found insufficient in Experiment 1 to result in spontaneous recovery in fetal responsiveness to the lemon solution.

This experimental protocol provides a means to discriminate habituation from peripheral mechanisms of response decrement. Specifically, if the decrement in fetal response following a series of infusions is due to peripheral mechanisms, then none of the dishabituation treatments should result in recovery of response at the time of the retest. However, if a central process such as habituation is involved, then the mint dishabituation treatment should increase the behavioural and cardiac responsiveness of fetuses to the lemon test. To analyse changes in fetal responses, motor activity and cardiac data were collected during the 30-sec periods following the ninth habituation trial, the dishabituation treatment, and the retest with lemon.

### Results and Discussion

As in Experiment 1, baseline levels of fetal HR were measured at four points during the observation session: (1) prior to the ninth habituation trial, (2) prior to the dishabituation treatment, (3) prior to the retest infu-

sion, and (4) 60 sec after the retest infusion. No significant changes in baseline levels of either motor activity or HR were evident across these four times of measurement. Absolute values of motor activity and HR were comparable with the reported results of Experiment 1. Because fetuses on Day 21 exhibited a higher resting HR than on Day 20,  $F(1, 88) = 36.2$ ,  $p < 0.001$ , cardiac responses to chemosensory infusions were expressed as HR change scores, as in Experiment 1.

The behavioural and cardiac responses of fetuses to the ninth exposure trial were compared in separate 2 (ages)  $\times$  6 (5-sec intervals) ANOVAs. There was no evidence that activity or HR changed as a function of time after infusion at either age. These results clearly replicate the data in Experiment 1 and confirm that fetuses exhibit a decrement in both motor and cardiac responses following a series of exposure trials in which lemon infusions are presented,

The motor activity and HR of fetuses exposed to different dishabituation treatments were compared in separate 2 (age)  $\times$  3 (treatment)  $\times$  6 (5-sec intervals) ANOVAs. The analysis of fetal activity indicated the significant Treatment  $\times$  5-sec Intervals interaction,  $F(10, 420) = 2.2$ ,  $p < 0.05$ . Fetuses exposed to mint were more active than NT controls or fetuses exposed to saline, and this effect was most evident 10–20 sec after infusion. The Treatment  $\times$  5-sec Interval interaction also was significant for changes in HR following dishabituation,  $F(10, 420) = 2.2$ ,  $p < 0.05$ . At both ages, fetuses exposed to mint exhibited significantly greater bradycardia during the first 10 sec after infusion than NT controls or saline-infused fetuses (see Figure 3).

The motor activity and HR response of fetuses to the retest with lemon was analyzed in separate 2 (age)  $\times$  3 (treatment)  $\times$  6 (5-sec intervals) ANOVAs. The analysis of motor activity indicated that the interaction of Age  $\times$  5-sec Intervals was significant,  $F(5, 420) = 2.4$ ,  $p < 0.05$ , suggesting that the temporal patterning of activity following lemon infusion differed as a function of age. This analysis also indicated the main effect of treatment,  $F(2, 84) = 4.0$ ,  $p < 0.05$ . Fetuses exposed to mint as a dishabituation treatment were more active after the lemon retest than NT or saline controls (see Figure 4). This finding indicates that mint infusion had the effect of dishabituating fetal motor activity. To analyse further the behaviour of fetuses in the mint group, a Pearson correlation was computed to assess the relationship between fetal activity after mint infusion and after lemon retest. No relationship between dishabituation and retest responses was evident on Day 20 [ $r(15) = -0.021$ , n.s.], but a significant positive relationship was apparent on Day 21,  $r(15) = -0.721$ ,  $p < 0.01$ . On Day 21, fetuses that were more responsive to the mint infusion during the dishabituation period were also more responsive to the retest with lemon.

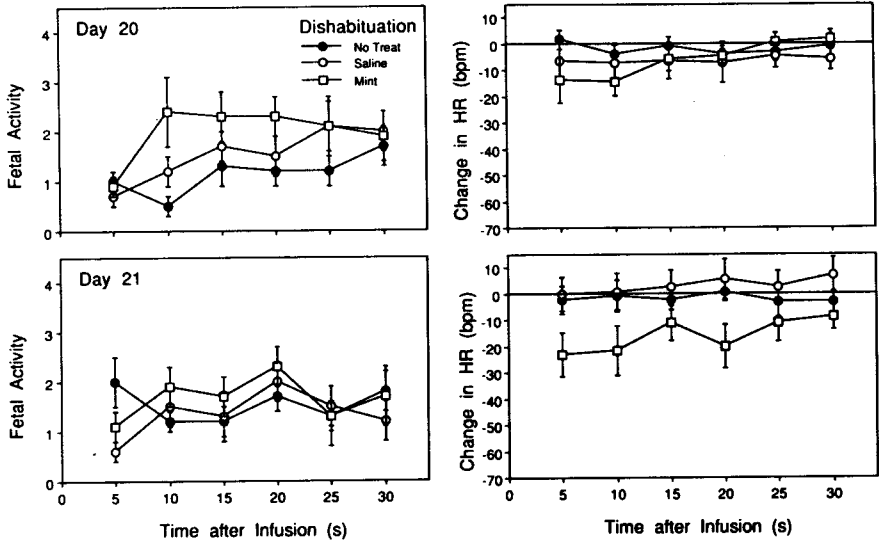


FIG. 3. Fetal motor activity and change in HR on Days 20 and 21 of gestation in response to the dishabitation treatments in Experiment 2.

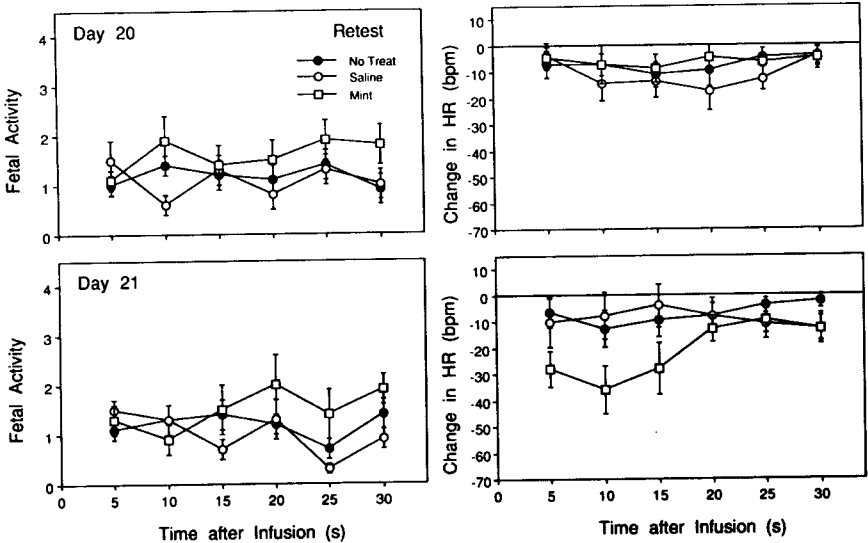


FIG. 4. Fetal motor activity and change in HR on Days 20 and 21 of gestation in response to the retest infusion of lemon following one of three dishabitation treatments in Experiment 2.

The ANOVA comparing changes in HR in response to the retest infusion revealed the significant interaction of Age  $\times$  Treatment  $\times$  5-sec Intervals,  $F(10, 420) = 2.0$ ,  $p < 0.05$ . Tests for simple interaction effects were conducted separately for each age of testing. On Day 20, the simple main effect of 5-sec Intervals ( $p < 0.05$ ) reflected the small but transient bradycardia evident in all three dishabituation groups upon retest with lemon. On Day 21, the Treatment  $\times$  5-sec Intervals interaction was significant ( $p < 0.01$ ). Fetuses exposed to mint during the dishabituation period exhibited a significantly greater bradycardia during the first 15 sec after retest infusion than did NT and saline-infused controls. Mint infusion had the effect of dishabituating cardiac responsiveness. Examination of the relationship between maximum bradycardia in response to mint and the retest with lemon revealed no correlation on Day 20 [ $r(15) = 0.002$ , n.s.] but a significant positive correlation on Day 21,  $r(15) = 0.569$ ,  $p < 0.05$ . Fetuses that expressed a cardiac response to mint infusion during dishabituation exhibited a greater bradycardia following the lemon test.

## GENERAL DISCUSSION

The results of both experiments confirm previous reports that initial exposure to a novel lemon stimulus evokes a pronounced response from fetuses that is evident in both motor activity (Smotherman & Robinson, 1990b) and heart rate (Smotherman, Robinson, Hepper, Ronca, & Alberts, 1991). Immediately after lemon infusion, motor activity increased two-fold, whereas HR was reduced 10–20% from resting levels before infusion. Over a series of nine exposures to the lemon stimulus, however, both the increase in activity and the bradycardia diminished in magnitude. By the last exposure, fetuses no longer exhibited motor or cardiac responses to infusion.

The demonstration of a response decrement to repeated stimulation implies habituation but does not exclude alternative processes such as receptor adaptation and effector fatigue (Jeffrey & Cohen, 1971). The dishabituation treatment involving mint infusion evoked both motor activity and cardiac responses only 60 sec after the last lemon exposure. Although mint may be processed by a different population of chemoreceptors, the expression of behavioural responsiveness to the mint stimulus rules out effector fatigue in the waning response to lemon.

Two minutes after the last lemon exposure trial, fetuses were retested with lemon. Subjects that received no treatment or a saline infusion during the dishabituation failed to exhibit motor and cardiac responses upon retest with lemon. The lack of response by these control fetuses is important, because it confirms that spontaneous recovery in fetal response did not occur. However, fetuses exposed to mint during dishabituation did express

an increase in motor activity (on both Days 20 and 21) and a significant bradycardia (Day 21 only) following the retest with lemon. The responsiveness of mint-infused fetuses to the lemon stimulus eliminates sensory adaptation as a possible mechanism underlying the waning of fetal response to repeated chemosensory stimulation.

Because the decrement in fetal responsiveness cannot be explained by peripheral effects on receptors or effectors, it must be attributed to a central process such as habituation (Thompson & Spencer, 1966). Closer examination of the responses of individual subjects to the mint infusion and subsequent retest with lemon confirmed the role of dishabituation in reinstating responsiveness to lemon. On Day 21, fetuses that were more responsive to mint exhibited more pronounced motor and cardiac changes following the retest infusion. Because the magnitude of response to the retest was contingent upon the responsiveness during dishabituation, these results support the conclusion of habituation in a chemosensory modality in the rat fetus on Day 21 of gestation. However, the inability to reinstate a cardiac response to lemon on Day 20 suggests a lack of congruence between changes in motor activity and HR in younger fetuses (Seigal, Sananes, Gaddy, & Campbell, 1987). No age-related differences in the motor response of fetuses to the retest were found, suggesting that habituation was responsible for the decrement in motor activity after repeated chemosensory stimulation. It appears that motor and cardiac systems are incompletely coupled on Day 20, and that assessment of central attentional processes such as habituation in very young subjects may be dependent upon the method of measuring response.

The principal conclusion of this study is that the rat fetus can alter its responses to sensory stimuli and that this change in responsiveness is mediated by the central process of habituation. This conclusion complements previous studies of exposure learning (Smotherman, 1982a; Hepper, 1988), classical conditioning (Smotherman, 1982b; Smotherman & Robinson, 1985), and associative learning (Robinson & Smotherman, 1991) during the prenatal period. In addition, it underscores the potential utility of the habituation paradigm for investigating ontogenetic changes in the organization of the central nervous system during the perinatal period. Even though the nervous system undergoes considerable development during the postnatal period, the findings of this study and other recent research clearly demonstrate that it is sufficiently well developed to support learning in the fetus. Because habituation can be investigated in an individual subject, in multiple sensory modalities, and with different, independent measures of behavioural response, it may be uniquely suited for probing the functional development of the CNS during the period before birth. Furthermore, if sensory experience is an important determinant of behavioural development before birth, it is essential to begin inquiry at this critical time, when ontogenetic trajectories are initiated.

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## L'habituation chez le fœtus de rat

Les fœtus de rat ont des réponses motrices et cardiaques à des stimulations chimico-sensorielles les vingt et vingt-et-unième jours de la gestation. La première expérience démontre que les fœtus ont une activité motrice générale augmentée et une décroissance du rythme cardiaque en réponse à une infusion orale initiale d'une solution au citron. Cependant, après une série de 9 expositions, on n'observe plus de réponses motrices ou cardiaques à l'infusion au citron, ce qui suggère l'existence d'un processus similaire à l'habituation. La réactivité réapparaît spontanément après une période de 3 à 9 minutes sans stimulation. Lors d'une seconde expérience, un protocole de déshabituación a été utilisé afin de différencier l'habituation, qui est une diminution de la réponse, d'origine centrale, de la fatigue des effecteurs, de l'adaptation sensorielle, et d'autres mécanismes périphériques pouvant provoquer une réactivité diminuée. Une seule infusion de menthe, consécutive à une série de neuf infusions de citron, suffit à provoquer à nouveau les réponses motrices fœtales en réaction au citron pendant les jours 20 et 21 de la gestation, mais ne ré-induit les réponses cardiaques que le 21ème jour. Les fœtus de rat s'habituent à une stimulation chimico-sensorielle répétée, ce qui souligne l'utilité du paradigme d'habituation pour la mesure du développement du SNC pendant la période périnatale.

## Habitación en el feto de rata

Los fetos de rata muestran respuestas motoras y cardíacas a la estimulación quemosensorial en los días 20 y 21 de la gestación. El primer experimento demostró que los fetos exhiben un incremento en actividad motora en general y una reducción en frecuencia cardíaca como respuesta a una infusión intra-oral de una solución de limón. Luego de una serie de nueve estimulaciones, sin embargo, desaparecen estas respuestas a la infusión de limón, sugiriendo la existencia de un proceso similar a la habituación. La responsividad se recupera espontáneamente al cabo de un período de 3 a 9 minutos sin estimulación. En un segundo experimento se utilizó un tratamiento de deshabitación para distinguir entre habituación, que es una reducción de la respuesta de origen central, y fatiga, adaptación sensorial, u otros mecanismos que pueden reducir la responsividad. Una sola infusión de menta luego de 9 estimulaciones con limón fue efectiva en re-instalar las respuestas motoras fetales en los días 20 y 21, pero produjo la recuperación de la respuesta cardíaca recién al día 21. Los fetos de rata se habitúan a la estimulación quemosensorial repetida, lo que sugiere la utilidad del paradigma de habituación para medir el desarrollo del sistema nervioso central durante el período perinatal.