

Heart Rate Response of the Rat Fetus and Neonate to a Chemosensory Stimulus

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SMOTHERMAN, W. P., S. R. ROBINSON, A. E. RONCA, J. R. ALBERTS AND P. G. HEPPER. *Heart rate response of the rat fetus and neonate to a chemosensory stimulus*. *PHYSIOL BEHAV* 50(1) 47–52, 1991.—Resting heart rate (HR) and HR responses of fetal and neonatal rats are described before and after intraoral infusion of isotonic saline or lemon solution. Stable measurements of resting HR were obtained for fetuses over the last three days of gestation (E19, E20, E21) and pups on the day of birth (P0) and four subsequent postnatal ages (P1, P3, P5, P7). Resting HR decreased significantly on P0 relative to the three prenatal ages and exhibited a linear increase thereafter. Variability in resting HR was pronounced on E21, decreased sharply after birth, and gradually increased through P7. Developmental changes in the HR response of fetuses and pups were evident following infusion of lemon. Fetal HR responses to lemon were characterized by bradycardia, which increased in magnitude through P1, diminished after P1, and eventually changed to tachycardia by P7. Both resting HR and HR responses to chemosensory stimulation point to the immediate perinatal period as a time of quantitative and qualitative change during sensory development.

Perinatal period Chemosensory stimulation Heart rate Fetal heart rate Sensory development

NEWBORN altricial rodents, such as rat pups, appear helpless but in fact possess a sophisticated behavioral repertoire that enables them to deal with the changing demands of their postnatal environment (4,20). The ingestive behavior associated with suckling is a clear example of early behavioral sophistication. Newborn rats can maintain an orientation relative to the mother, locate and attach to a nipple, and suckle to elicit milk letdown (6). Aversive responses to appropriate stimulation, while not ordinarily expressed in the nest environment, are also evident as early as one day after birth. Certain components of adult aversion reactions, such as gaping, forelimb flailing, and suppressed intake can be evoked from one-day-old rat pups exposed to strong quinine or acid solutions (17). Behavioral findings that young mammals are highly responsive to olfactory or gustatory

stimuli (10, 21, 39) are complementing a growing neurobiological literature concerned with the early development and functional activity of chemosensory systems (7,24). Providing perinatal subjects with chemosensory stimuli has proven effective in unmasking early behavioral organization or competence.

The developmental origin of chemosensory-evoked behavioral responses recently has been traced to the prenatal period. By day 19 of a 21.5 day gestation, rat fetuses exhibit a general increase in motor activity in response to a wide range of chemical stimuli, including milk and lemon (34). Specific behavioral responses to milk and lemon are expressed 24 h later (33). Intraoral infusion of milk to 20-day-old fetuses elicits a stereotypic stretch response that closely resembles the behavior expressed by pups receiving milk at the nipple (9,22). Exposure to

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a lemon solution at this age elicits the equally distinctive behavior of facial wiping, which appears to correspond to components of adult aversion and grooming sequences (11,12). This behavioral evidence demonstrates that the fetus can detect, distinguish, and selectively respond to different classes of chemical stimuli in utero.

Functional expression of chemosensory-evoked behavior by fetuses and neonates implies continuity over the perinatal period. However, reliance on motor performances to measure sensory responsiveness during the perinatal period is complicated by changes in the physical environment associated with birth. For instance, the tendency for newborn rats to express the facial wiping response after lemon infusion is contingent upon testing in an environmental context that permits forelimb-facial contact (e.g., immersion in a buoyant fluid) (36). Asking specific questions about behavioral continuity is complicated because experimental procedures and behavioral measures appropriate for one period of development may be inappropriate at other ages (3,40). Objective resolution of issues of continuity necessitates that methods be adopted that can be applied at disparate points in development.

Phasic changes in HR have figured prominently in psychophysiological research on postnatal subjects as a measure of sensory responsiveness. Cardiac responses of developing organisms can reveal early sensory competence (15, 30, 42) and reflect differential experience with stimuli (23, 31, 41). Because techniques for recording fetal cardiac reactivity can be employed across a great diversity of species (2,18), it is especially promising as a tool in the study of sensory development. Further, HR can be recorded from both pups (29) and fetuses (35). In a recent report, HR was successfully employed to measure chemosensory responsiveness in term and caesarean-derived preterm rat pups (28), corroborating the utility of this metric in studies conducted during the perinatal period. In the present study, the cardiac responsiveness of rat fetuses and rat pups to infusions of chemosensory fluids was investigated.

METHOD

Subjects

Adult female Sprague-Dawley rats (Charles-River Labs., Wilmington, MA) were maintained under conditions of constant room temperature (22°C) on a 12-h light:dark cycle (lights on at 0700). Food and water were freely available. Females were bred with Long-Evans male rats to produce animals for the experiments. Daily vaginal smears during the period of breeding were examined to date conception (the presence of sperm in a smear was designated as the start of pregnancy, E0). Breeding females were housed in groups of three in polycarbonate cages (33 × 38 × 10 cm). For pups to be tested after birth, females were re-housed individually one day prior to parturition. Animals were maintained and used in accordance with the NIH Guide for the Care and Use of Laboratory Animals (PHS Publication No. 86-23).

Each fetal or neonatal subject was fitted with cardiac leads and tested at only one age. Resting HR and HR variability were measured in a total of 486 fetuses and pups. The large sample sizes tested at each age reflects the fact that collection of resting HR data has been a standard protocol in our laboratory. The sample sizes tested at each age (E19, N=40, E20, N=42, E21, N=60, P0, N=81, P1, N=100, P3, N=100, P5, N=30 and P7, N=33) were collected from the preinfusion period of subjects in the present study as well as data from several normative studies conducted in our laboratory. HR responses to chemosensory infusion were measured in a total of 172 fetuses and pups

(E19, N=23, E20, N=25, E21, N=23, P0, N=20, P1, N=19, P3, N=20, P5, N=20 and P7, N=22). To eliminate the confounding of treatment with litter effects, no more than one animal from a given mother was tested in a particular treatment condition. A total of 116 dams provided fetal and neonatal subjects in this study.

Prenatal Preparation

To record fetal HR, pregnant females were prepared for testing of fetuses on embryonic day E19, E20, or E21 according to procedures fully described by Smotherman, Richards and Robinson (32). Pregnant females were briefly anesthetized using ether and spinal anesthesia was induced by injection of 100% ethanol between the first and second lumbar vertebrae. This procedure eliminates sensation in the lower body. The female was then placed in a Plexiglas holding apparatus in a bath of buffered isotonic saline (37.5°C) immersing the lower body. The uterus was then externalized by midline laparotomy. The mother and fetuses were left for 20 minutes to recover from the ether anesthesia. Subject fetuses were delivered from the uterus and amniotic sac into the bath, but remained attached by means of the umbilical cord to the placenta, which remained within the uterus. The coloration of the umbilical cord and fetus was visually assessed during the experiment to ensure that the fetus remained fully oxygenated.

Postnatal Preparation

Females and offspring were left undisturbed after birth (vaginal delivery) until the time for testing on postnatal day P0, P1, P3, P5, or P7. Experiments conducted on the day of birth (day P0) involved testing pups prior to suckling experience. Litters were separated from dams and placed in a warm humid incubator (32.5°C), where they remained for 30–60 min prior to testing. All testing occurred between 1300–1700. Postnatal testing was conducted after placement of individual subjects in a small (12 × 12 cm) plastic-floored testing arena maintained at 32.5°C.

HR Recording

Fetuses and pups were fitted with paired cardiac leads fashioned by stripping the terminal insulation from #36 nickel-chrome wire. The tips of the two leads were bent to a sharp angle, coated with lidocaine, and inserted under the skin ventrally (overlying the sternum) and dorsally (overlying the thoracic vertebrae). The leads were connected to a Grass model 79 polygraph, which amplified the EKG signal and provided a permanent strip record of individual heart beats (35). This strip record was divided into successive 5-s periods and the number of beats counted to calculate HR over the session. Recording commenced after a delay of 2 min in the testing environment.

Cannulation

Controlled presentation of stimuli into the mouth of individual rats was accomplished through the use of an intraoral cannula (14). The cannula consisted of a length of PE-10 polyethylene tubing (outer diameter=0.61 mm) inserted through the midline of the lower jaw with the flanged tip resting on the dorsal surface of the tongue. The position on the tongue was equivalent to the anterior placement described by Kehoe and Blass (19). The free end of the cannula was connected by way of a length of PE-50 tubing to a micrometer syringe. This system enabled precise infusion ($\pm 1 \mu\text{l}$) of a solution to the animal without otherwise interrupting ongoing activity. Infusions were

delivered in a 1–2-s pulse. Animals at ages E19, E20, E21 received 20 μ l infusions, pups at ages P0, P1 and P3 received 30 μ l infusions, pups at ages P5 and P7 received 40 μ l and 50 μ l infusions, respectively. Solutions delivered by intraoral infusion consisted of isotonic saline or lemon extract presented in an isotonic saline carrier. The lemon solution was prepared by mixing one part of pure lemon extract (Schilling brand) in three parts saline, centrifuging and removing the supernatant oil (33). Test solutions were delivered at ambient testing temperature: 37.5°C for fetuses and 32.5°C for pups.

Data Analysis

HR was continuously recorded for 55 seconds before infusion and 30 seconds after infusion. All fetuses and pups included in these samples were fitted with an intraoral cannula, but did not receive an infusion during the collection of resting HR data. These data were analyzed in a one-way (8 ages) between-subjects ANOVA. HR was calculated for each of the 11 5-s intervals before infusion. HR scores in successive 5-s intervals were compared to measure HR variability. Transitions between intervals in which HR changed by more than 5% were considered instances of high HR variability.

To measure cardiac response to infusion, the postinfusion period was divided into six 5-s intervals to permit analysis of cardiac responsiveness to stimulation. For each subject, a series of change scores was calculated as the difference between absolute HR during the 5-s interval and the baseline HR expressed during the 5-s interval immediately preceding the infusion. Change scores were compared in a three-factor (8 ages \times 2 stimuli \times 6 intervals) repeated-measures ANOVA. The purpose of this analysis was to characterize the temporal pattern of HR response at different ages following saline or lemon infusion.

A second analysis was employed to determine whether a significant change in HR occurred after infusion. In this analysis, the 5-s interval exhibiting the largest change in HR was defined as the Peak Interval. Data from the Peak Interval were compared in a two-factor (8 ages \times 2 stimuli) ANOVA. Comparison of the peak change in HR to baseline HR permitted identification of ages at which lemon or saline infusion evoked significant change in HR. This comparison was performed by constructing separate 99% confidence intervals around preinfusion baseline HR for each age. HR change scores that lay more than one standard error above or below the confidence interval were interpreted as evidence of significant cardiac response to infusion. Correlation coefficients were calculated to determine whether the magnitude of cardiac response was influenced by the baseline HR of each subject (26).

RESULTS

Resting HR in the Fetal and Neonatal Rat

Analysis of resting HR indicated the significant effect of age, $F(7,478)=73.0$, $p<0.01$ (Fig. 1). Post hoc comparison of means by the method of Newman-Keuls (p values for all comparisons <0.01) (43) indicated that prenatal resting HR was lower on E19 than on E20 and E21. After birth, HR increased significantly from P0 through P3. HR did not exhibit further increase between P3 and P7. At the same postconceptional age, pups (P0) exhibited a significantly lower resting HR than fetuses (E21).

Resting HR appeared to be more variable at some ages than at others. The percentages of all transitions that exhibited high variability over the perinatal period are depicted in Fig. 2. Overall, HR variability during the perinatal period differed sig-

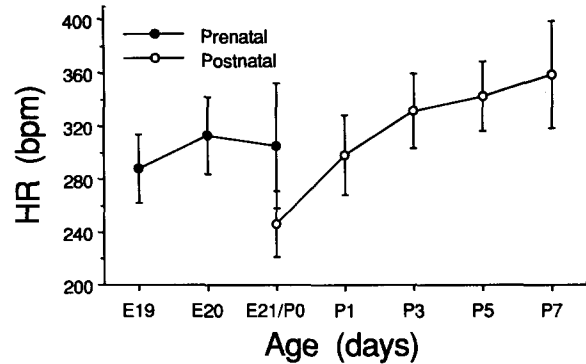


FIG. 1. Resting heart rate (mean \pm SD) of rat fetuses and pups. While virtually the same postconceptional age, resting HR is significantly lower in pups on the day of birth (P0) than fetuses at term (E21).

nificantly as a function of age, $\chi^2(N=486, df=7)=48.4$, $p<0.001$. Fetuses at term (E21) exhibited very high HR variability from one 5-s interval to the next compared to younger fetuses or newborn pups. During the postnatal period, HR variability increased with advancing age.

Perinatal Cardiac Response

The ANOVA that compared subjects infused with saline or lemon at one of eight perinatal ages indicated the significant three-way interaction of chemosensory stimulus by age by interval after infusion, $F(35,780)=2.7$, $p<0.001$ (Fig. 3). Tests for simple interaction effects were conducted separately for saline-infused and lemon-infused groups to assess age-related changes in the temporal pattern of cardiac response to each stimulus [(43), p. 350]. A main effect of age was evident for subjects in the saline-infusion group, $F(7,77)=4.3$, $p<0.001$. Further post hoc comparisons by the method of Newman-Keuls indicated that subjects on the day of birth (P0) were more responsive to saline infusion than at any other prenatal or postnatal age, including same-age fetuses on E21 (p values <0.01 for all comparisons). For subjects that received the lemon stimulus, the interaction of age by interval after infusion was significant, $F(35,395)=5.2$, $p<0.001$. Cardiac responsiveness to the lemon stimulus emerged during the prenatal period in the form of HR deceleration.

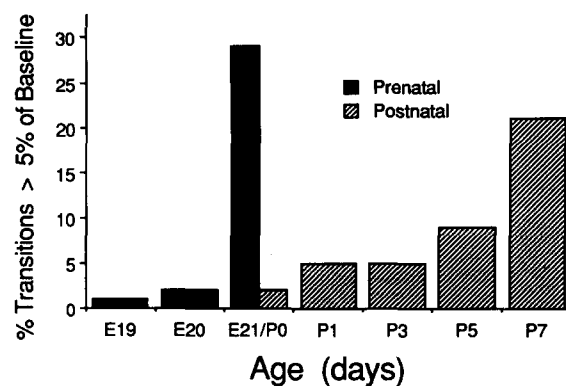


FIG. 2. Variability in resting heart rate of rat fetuses and pups. Bars represent the percentage of all transitions between consecutive 5-s intervals that differed by more than 5% of resting HR. Data are plotted separately for prenatal (E19–E21) and postnatal (P0–P7) subjects.

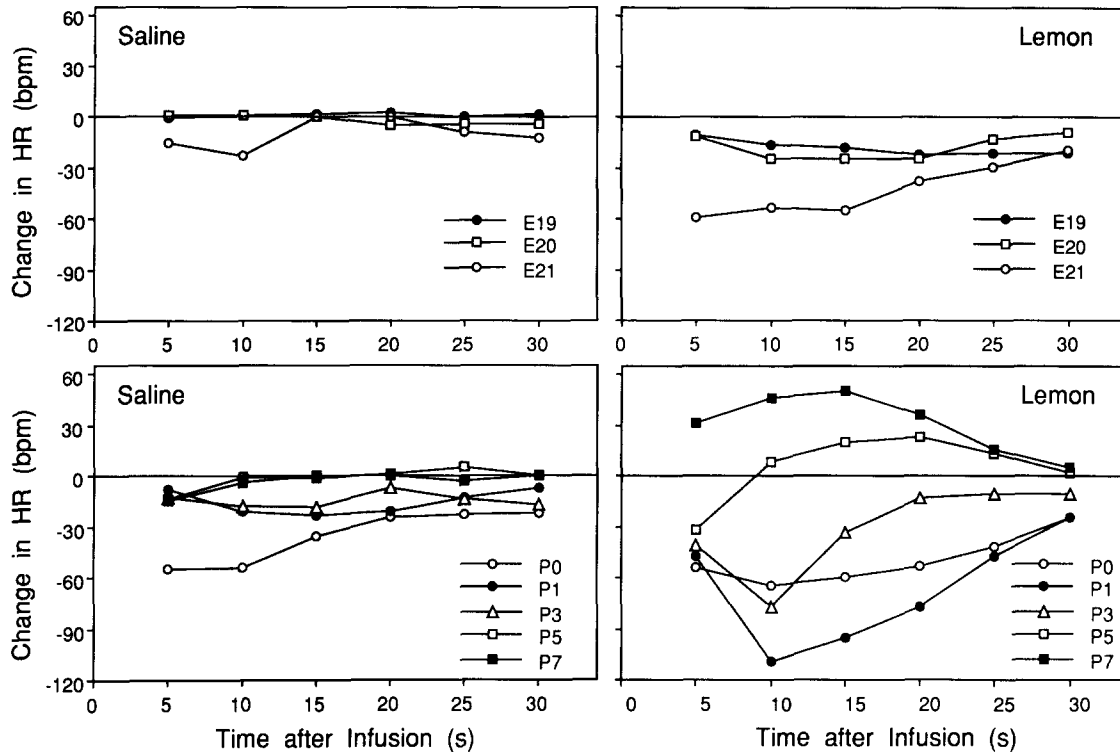


FIG. 3. Cardiac responses of rat fetuses and pups to intraoral infusions of saline or lemon. Points represent mean change in HR during the 30 s following infusion. The zero line in each graph depicts resting HR during the 55-s interval prior to infusion.

Mean HR responses to lemon infusion suggested a developmental shift from bradycardia to tachycardia among postnatal subjects. To further characterize this developmental trend, HR changes in individual subjects were examined and classified as pure bradycardia, biphasic (initial bradycardia giving way to tachycardia), or pure tachycardia. A change in HR exceeding $\pm 5\%$ of baseline was used as the criterion for classifying HR responses. The incidence of these three patterns of HR response varied significantly with age, $\chi^2(8) = 44.4, p < 0.001$ (Table 1). The incidence of pure bradycardia declined steadily with age from P0 (100%) through P7 (9%). Pure tachycardia was first evident on P5 and was expressed by 82% of subjects on P7. On P5, although some subjects exhibited pure bradycardia or tachycardia, the modal HR response was biphasic.

Peak change in HR during the 30 s following infusion varied as a function of age and stimulus, $F(7,156) = 5.6, p < 0.001$ (Fig. 4). Among saline-infused fetuses, HR was significantly reduced

from resting levels on days P0 and P1, with the greatest bradycardia occurring on the day of birth. Two developmental trends were evident among fetuses that received lemon infusion, consisting of bradycardia of increasing magnitude from E19 through P1, a reduction of bradycardia through P5, and a significant tachycardia on P7. All of the peak changes in HR evoked by lemon infusion represented significant deviations from baseline except on day P5. Peak HR responses to saline and lemon infusion differed from one another on E21, P1, P3 and P7, but did not differ on the day of birth or on P5. The expression of bradycardia, tachycardia and biphasic HR changes by different subjects on P5 masks the overall responsiveness of these subjects as a group. In fact, subjects that received lemon infusion were responsive when peak HR response of P5 subjects was calculated as an absolute value; the absolute peak change was 70.8 ± 10.2 bpm, which differed significantly from baseline HR. Thus fetuses and pups exhibited significant HR responses to lemon infusion at all ages tested.

There was no evidence that the magnitude of HR change was influenced by baseline HR during the minute preceding infusion (Pearson $r = .02, N = 93, p > 0.10$). A lack of significant correspondence between resting HR and cardiac response also was found when comparing fetuses (E19–E21) and pups (P0–P7) separately (fetuses: $r = -.34, N = 37, p > 0.10$, pups: $r = .18, N = 56, p > 0.10$).

TABLE 1

PATTERNS OF HEART RATE CHANGE FOLLOWING LEMON INFUSION IN POSTNATAL SUBJECTS

	P0	P1	P3	P5	P7
Bradycardia	10	9	6	2	1
Biphasic (bradycardia and tachycardia)	0	1	4	5	1
Tachycardia	0	0	0	3	9

Entries represent number of subjects at each age that exhibit each pattern of HR response.

DISCUSSION

Heart rate provides a useful metric for assessing responsiveness to sensory stimulation during the perinatal period. The techniques employed provide accurate and stable measurements of HR during both the prenatal and immediate postnatal periods.

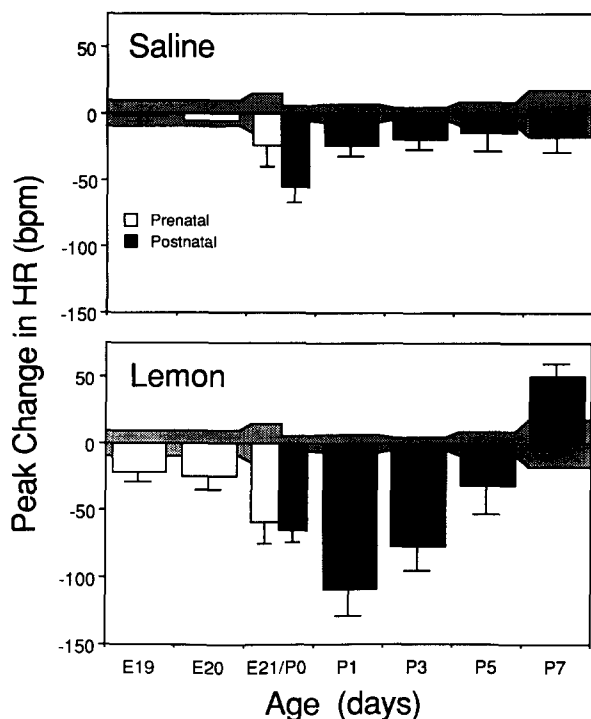


FIG. 4. Peak change in HR of rat fetuses and pups following infusion of saline (top panel) or lemon (bottom panel). Bars represent the HR change score (mean \pm SE) of greatest magnitude during the 30 s following infusion. Data are plotted separately for prenatal (E19–E21) and postnatal (P0–P7) subjects. The zero line represents resting HR during the 55 s prior to infusion, with the shaded area depicting the 99% confidence limit around this resting HR at each age.

Values for resting HR on P0 and P1 replicate HR measurements reported by Ronca and Alberts (28), and data for older pups are similar to values reported by Wigal, Dailey and Amsel (42). The resting HR of fetuses that retain placental-uterine attachment was markedly higher than caesarean-derived preterm pups tested at the same postconceptional age (28), but accorded well with recent HR measurements obtained from rat fetuses by ultrasonography (25) and with older data from rat fetuses adjusted for differences in body temperature (1). The increased variability in HR on E21 was noteworthy, and probably was due to uterine contractility in advance of labor. The gradual increase in postnatal HR variability (most evident on P3–P7) may have been due to differences in overall motor activity exhibited by pups.

Chemosensory infusion was effective in eliciting cardiac responses from both fetal and neonatal rats. The pattern of these HR responses varied as a function of age. Bradycardia in response to lemon infusion was most pronounced at term and during the first 24 hours after birth. Although resting HR also was found to vary with age, there was no evident relationship between resting HR and the magnitude of cardiac response after infusion. This finding contrasts with the data of Ronca and Alberts (28), who reported that HR responses of caesarean-derived preterm rat pups covaried with resting HR; pups with greater resting HR exhibited less pronounced bradycardia in response to lemon infusion. This discrepancy may reflect the impaired physiological status of preterm-delivered pups, which suggests that baseline HR may be relevant in interpreting sensory-evoked HR responses of premature subjects.

Other studies reporting cardiac responses during the perinatal

period have focused on older pups [e.g., (42)], have averaged response data over a large number of stimulus presentations (8), or have tested pups after varying periods of maternal and food deprivation (16). What is striking about the present results, in light of published data, is the magnitude of HR change evoked by a single exposure to a novel chemosensory stimulus. For example, Dailey and Amsel (8) report a maximal HR deviation from baseline of 2–3% averaged over 60 milk infusion trials in 8-day-old pups. Small changes in HR following exposure to odorants also have been reported in rats on P2–P3 (5,23). In contrast, this study found a 25% change in HR on P1 [cf. (28)]. This pronounced cardiac response was evident before birth as well, as illustrated by a 20% HR deviation from baseline in 21-day-old fetuses exposed to a single lemon infusion.

Pups on P0 exhibited bradycardia to both lemon and saline infusion, an event that constituted their first exposure to a fluid in the mouth after the onset of breathing. Introduction of a fluid in the upper respiratory tract is known to evoke bradycardia in newborn lambs (13). Bradycardia evoked by infusion of a lemon solution is associated with respiratory slowing in newborn rats (28). Evidence from fetuses and pups suggests that behavioral responses after infusion are evoked by chemosensory properties of the lemon solution (37). However, the HR response to saline, which is evident in newborn pups (P0) but is not expressed by fetuses of the same postconceptional age (E21), diminishes in magnitude over the first few days of postnatal life (P0–P3). This developmental change in HR response to saline may reflect the pup's increasing ability to maintain adequate ventilation while processing a fluid in the oral cavity. During the first hours after birth, the newborn must initiate air breathing and coordinate this activity with suckling. These findings suggest the hypothesis that the newborn's cardiac responsiveness to fluid stimuli is influenced by the parallel development of breathing, suckling, the interaction of these activities, and perhaps other processes involved in the transition between prenatal and postnatal life.

The cardiac response to saline infusion and the suppression in resting HR that are evident on the day of birth probably reflect the physiological adaptation of the newborn rat to an air-breathing existence. In contrast, an inflection point is apparent on P1 between two developmental trends in cardiac response to lemon infusion. Prior to P1, the bradycardia evoked by a lemon stimulus increases in magnitude with advancing age. After P1, the pattern of cardiac response to chemosensory stimulation gradually shifts to a biphasic response comprising both bradycardia and tachycardia (P5) and ultimately to a uniform tachycardia (P7). Because the inflection point in cardiac response to lemon infusion is delayed relative to the time of birth, it seems unlikely to be due to the physiological adaptation of the newborn rat to the postnatal environment. Cardiac deceleration or acceleration in response to stimulation, as demonstrated in the present study, traditionally has been related to central sensory processes, such as the orienting response and defensive response (27,38). Given the differences in stimuli and developmental gradation from bradycardia to tachycardia, the relationship of perinatal HR responses to organizing concepts of sensory processing remains uncertain, suggesting the need to employ multiple, independent measures of sensory responsiveness in fetuses and neonates.

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