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Response of the Rat Fetus to Acute Umbilical Cord Occlusion: An Ontogenetic Adaptation?

WILLIAM P. SMOTHERMAN[1] AND SCOTT R. ROBINSON

Laboratory for Psychobiological Research, Departments of Psychology and Zoology Oregon State University, Corvallis, OR 97331

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SMOTHERMAN, W. P. AND S. R. ROBINSON. *Response of the rat fetus to acute umbilical cord occlusion: An ontogenetic adaptation?* PHYSIOL BEHAV **44**(1) 131–135, 1988.—Occlusion of the umbilical cord on days 19, 20 or 21 of gestation results in a stereotypic behavioral response by the rat fetus. The intensity of this response is diminished in older fetuses. Abrupt changes in fetal heart rate accompany the behavioral response to hypoxia. Changes in fetal activity and heart rate appear to be disassociated, however, because bradycardia immediately after umbilical cord occlusion is more pronounced in older fetuses. The possibility that these behavioral and cardiac responses to hypoxia are ontogenetic adaptations restricted to the prenatal period is discussed.

Rat fetus   Hypoxia   Motor behavior   Heart rate   Umbilical cord   Ontogenetic adaptation

PREVIOUS studies have documented that the 20-day-old rat fetus exhibits a stereotypic behavioral response to acute hypoxia resulting from umbilical cord occlusion (3,17). This behavior comprises three distinct phases. In the first phase, fetal activity is sharply reduced. In the second phase, fetal movement increases four- to five-fold over spontaneous rates of activity. In the third phase, movement again diminishes to near quiescence. Characteristically, the entire behavioral sequence occurs with a consistent time course, the third phase beginning 60–90 sec after the onset of umbilical cord occlusion.

Acute fetal hypoxia, induced by complete or partial compression of the umbilical cord, is among the most important stresses to which the fetus is exposed during gestation (9) and has been suggested as an important cause of brain damage during the late prenatal period (11). Fetuses are at risk of transient umbilical cord occlusion even in normal pregnancies, when changes in the intrauterine environment and the position of the fetus (18) increase the chance of cord compression. For example, the fetal rat on day 19 (of 21-day gestation) is surrounded by a maximum volume of amniotic fluid, and therefore should experience little risk of cord compression. By comparison, the fetus on day 21 is more than twice as large as on day 19 but is surrounded by only a fifth the volume of fluid; the risk of cord compression therefore should be greater. The implication of these facts is that the prenatal environment is not uniformly protective of the fetus and can pose occasional hazard to fetal well-being. Further, the likelihood that a fetus will encounter such suboptimal environmental conditions in utero varies with gestational age.

The principal aim of this study was to examine the behavioral response of rat fetuses to acute hypoxia at three gestational ages, days 19, 20 and 21, which are associated with markedly different intrauterine environments. A second aim was to simultaneously monitor fetal heart rate, a physiological measure that changes under various forms of fetal stimulation (14) and is likely to covary with the rapid behavioral response to hypoxia. Previous approaches to the study of fetal hypoxia, which generally have focused on physiological changes measured over tens of minutes after manipulation of maternal oxygen availability, have documented variation in cardiovascular activity during hypoxia (1,4). However, variation in fetal heart rate during the first minutes after the onset of acute hypoxia, which comprise the entire time course of the behavioral hypoxic response in the rat fetus, has not been reported.

METHOD

Subjects

Adult female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were maintained under conditions of constant room temperature (22°C), under a 12 hr light:12 hr dark photoperiod with lights on at 0700. Food and water were continuously available. Females were bred to Long-Evans males; this cross produces large litters of vigorous offspring ideally suited to prenatal study. Daily

[1]Requests for reprints should be addressed to William P. Smotherman, Ph.D., Department of Psychology, SUNY Binghamton, Binghamton, NY 13901.

vaginal smears during the period of breeding were examined to identify the date of conception (the first sperm-bearing smear was designated day 0 of gestation). A total of 32 pregnant rats each provided one fetus that was tested on day 19 (N=12), day 20 (N=10), or day 21 (N=10) of gestation (birth occurs about day 21.5). Animals were maintained and used in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23).

Preparation of the Pregnant Female

The methods used for preparation of the pregnant rat for fetal observation are standard procedures in our laboratory and have been described in detail (15,19). Briefly, under ether anesthesia, a chemomyelotomy was performed to chemically transect the rat's spinal cord between the first and second lumbar vertebrae, eliminating afferent stimuli from the lower body. The prepared female was secured in a holding apparatus and her abdomen and hindlimbs immersed in a bath of isotonic saline (Locke's solution) maintained at 37.5°C. The rat's uterus was externalized through a midline laparotomy and suspended just beneath the surface of the bath. A period of 15–20 min elapsed between the time of surgery and observation.

Preparation of the Subject Fetus

At the beginning of the observation period one subject fetus was delivered from the uterus and its amniotic sac into the bath, preserving the attachment of the placenta to the uterus. The fetus was fitted with a pair of cardiac leads, which were fashioned by stripping the terminal insulation from No. 36 nickel-chrome wire. The tips of the two leads were bent to a sharp angle and inserted under the skin ventrally (overlying the sternum) and dorsally (overlying the thoracic vetebrae). The leads were connected to a Grass model 79 polygraph, which amplified the EKG signal and provided a permanent strip record.

Measurement of Behavioral and Cardiac Response

Fetal behavior and heart rate were continuously recorded for the subject fetus during a 180-sec observation session. The first 55 sec of the session provided baseline measurements of spontaneous fetal activity and resting heart rate. Between 55–60 sec into the session, the umbilical cord was occluded with the application of a microvascular clamp (RS-6470, Roboz Surgical Instrument Co., Washington, DC) 5–10 mm from the fetus's abdomen (17). Attachment of the clamp applied 10–15 g of compression pressure, which was sufficient to eliminate umbilical arterial and venous circulation during the final 120 sec of the observation session.

Behavioral observation consisted of entering each instance of movement of the head, mouth, forelegs, rearlegs or body trunk of the fetus into a real-time event recorder (13,16). The reliability of this data recording protocol is consistently high, in excess of 0.90 (both between rater and between successive observations by the same rater). The sum of these individual movements provided a measure of overall fetal activity. Polygraph records of fetal cardiac activity were marked into intervals and individual heart beats counted to provide a measure of fetal cardiac output. Both behavioral and heart rate data are reported for the 15-sec interval preceding and the eight successive 15-sec intervals following application of the microvascular clamp.

RESULTS

Behavioral Response

In this study, overall fetal activity during the 15-sec interval preceding umbilical cord occlusion amounted to 4.6±0.4 movements on day 19, 4.6±0.4 on day 20, and 4.4±0.5 on day 21. These data are consistent with our previous reports of spontaneous activity of unmanipulated rat fetuses (15,16). Therefore, these results supported our subjective impression during observation sessions that the presence of the cardiac leads did not influence overall levels of fetal activity.

Fetal activity changed abruptly after application of the umbilical cord clamp (at t_0) (Fig. 1a). A two-way repeated measures analysis of variance (ANOVA) comparing overall fetal activity revealed the significant interaction of Age × Interval after occlusion, $F(14,203)=4.7$, $p<0.01$. Fetuses were generally most active on day 19 and least active on day 21. However, activity was distributed differently over the eight intervals at each age. At all three ages, activity was suppressed between t and t_{30}. An abrupt increase in activity was evident at the two younger ages, with peak activity occurring at t_{60} on day 19 and t_{45} on day 20. On days 19 and 20, activity declined after t_{60}. On day 21, this pattern of sharp increase and decline in activity was less evident.

The interaction of Age × Interval after occlusion was also significant for movements involving the body trunk of the fetus, $F(14,203)=7.4$, $p<0.01$; Fig. 1b. Most of these trunk movements involved vigorous lateral felxion ("curling") that coincided with the peak of fetal activity. For all three gestational ages, the temporal distribution of trunk movements closely paralleled the pattern described for overall fetal activity. The interaction was also significant for head movements, $F(14,203)=2.9$, $p<0.01$; Fig. 1c. On days 19 and 20, head movements reached their highest incidence subsequent to the peak in trunk activty and occurred during the secondary decline in overall activity (t_{60}–t_{75}). Many of these head movements, especially those that occurred after the peak in overall activity, assumed the form of a rostral extension ("tossing"). Foreleg movements did not vary with gestational age, but for all ages showed a temporal pattern of decrease, increase and decrease that corresponded to changes in overall activity, $F(7,203)=8.6$, $p<0.01$; Fig. 1d. Most foreleg movements appeared uncoordinated, but some that occurred after the peak in overall activity involved synchronous rostral extension of both forelegs in a posture that was maintained for a second or longer. Rearleg (Fig. 1e) and mouth (Fig. 1f) movements were relatively infrequent and did not differ as a function of Age or Interval after occlusion.

Subjectively, the behavior exhibited by rat fetuses during the peak of activity appeared highly energetic, particularly in comparison with the character of spontaneous (preocclusion) activity. Lateral trunk curls typically were directed alternately to left and right, so changes in fetal posture were rapid and pronounced. On occasion, fetal hyperactivity resulted in removal of the microvascular clamp from the umbilical cord and forced termination of the test session. Behavioral and cardiac data from these fetuses were not included in this report.

Heart Rate Response

A one-way ANOVA compared baseline HR during the 15-sec interval preceding umbilical cord occlusion. No significant difference was indicated among fetuses of different ges-

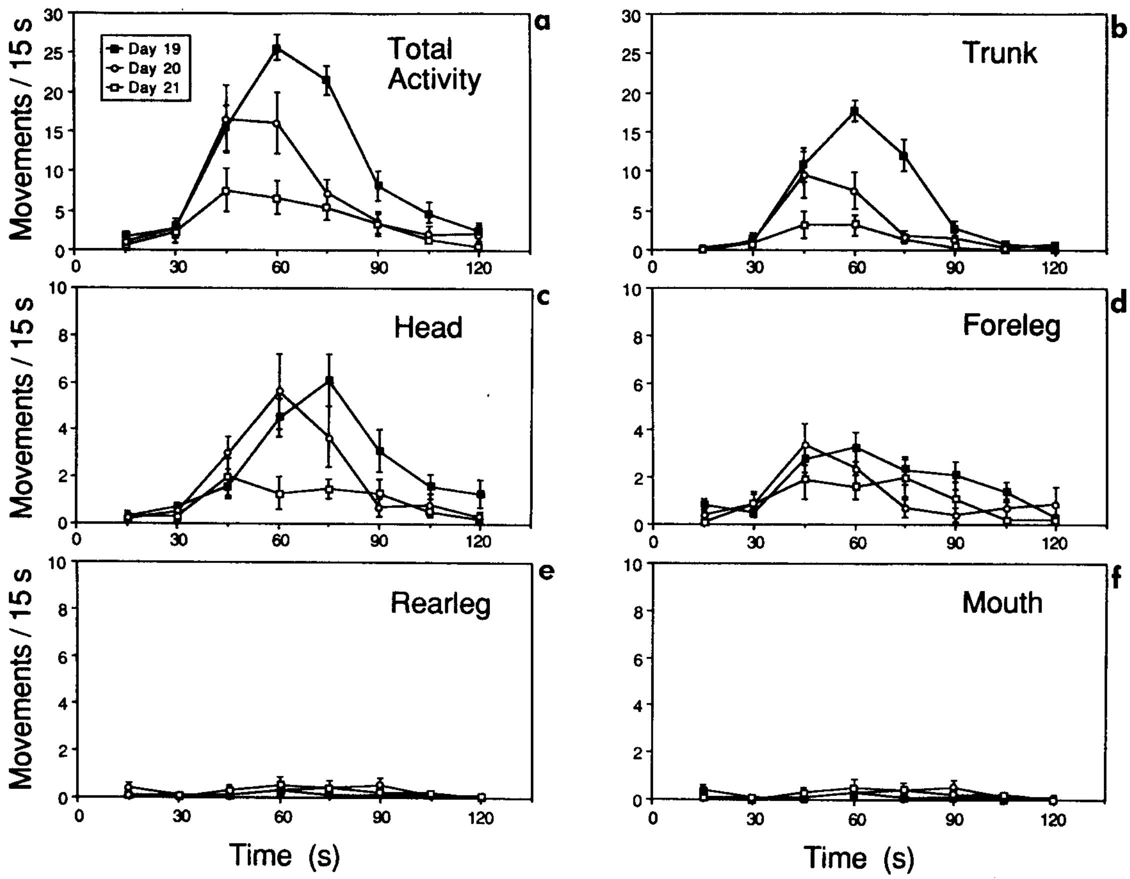

FIG. 1. Overall activity and incidence of individual movement categories of rat fetuses on days 19, 20 and 21 of gestation following acute umbilical cord occlusion (clamp on at t_0). Points represent mean number of fetal movements and vertical lines represent SEM. Mean total activity prior to umbilical cord occlusion on days 19, 20 and 21 were 4.6, 4.6, and 4.4 movements/15 sec, respectively. Ordinate scales for total activity and trunk movement differ from other categories.

tational age (mean HR in bpm: day 19=298.8±4.8; day 20=312.8±13.8; day 21=291.6±16.0) $F(2,29)=0.78, p>0.05$. Both within and between individuals, baseline HR exhibited relatively low variability. Therefore, the HR monitoring technique employed in this study appears to be suitable for simultaneous recording of fetal HR and behavioral activity (14). To be conservative, however, any influence of baseline variation on HR reactivity was eliminated by expressing HR during the eight 15-sec intervals following application of the clamp as a change score. To calculate this change score; the absolute HR during each 15-sec interval following umbilical occlusion was subtracted from the HR of the same pup during the 15-sec baseline interval.

Systematic changes in HR following occlusion of the umbilical cord were evident (Fig. 2). A two-way repeated measures ANOVA revealed the significant interaction between Age and Interval after occlusion, $F(14,203)=4.7$, $p<0.01$. At all three ages, HR decreased after application of the clamp. During the first postclamp interval, the decrease in HR was most pronounced on day 21 and least on day 19. The magnitude of initial bradycardia amounted to a 12% reduction from baseline HR on day 19, 17% on day 20, and 32% on day 21. Across the eight intervals fetal HR continued to decline, and by the eighth postclamp interval the magnitude of bradycardia was virtually the same across the three ages,

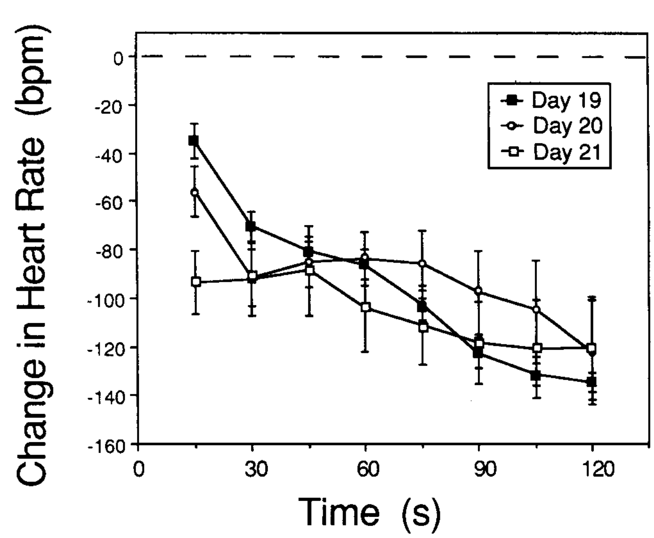

FIG. 2. Change in heart rate of fetuses on days 19, 20 or 21 following acute umbilical cord occlusion. The dashed line represents mean heart rate during the 15-sec interval immediately preceding application of the umbilical clamp (see text for details). Points represent mean values and vertical lines represent SEM.

amounting to a 45% reduction from baseline HR on day 19, 39% on day 20, and 40% on day 21. Therefore, fetuses of different gestational age could be distinguished only by the magnitude of the initial bradycardia.

DISCUSSION

The findings of this study confirm the existence of a consistent, rapid, and stereotypic response of the fetal rat to occlusion of the umbilical cord. The behavioral data replicate our previous description of the hypoxic response of day 20 rat fetuses (17). At all three ages, fetal activity diminished after cord occlusion, exhibited an increase above preocclusion levels, and again diminished. At the peak of activity (t_{45}–t_{60}, Fig. 1a), 92% of day 19 fetuses, 70% of day 20 fetuses and 60% of day 21 fetuses exhibited at least a fourfold increase over spontaneous rates of movement. This fact emphasizes both the consistency and intensity of the behavioral response to hypoxia.

The fetal hypoxic response also differs qualitatively from spontaneous fetal activity. Although spontaneous activity often is vigorous, it seldom approaches the magnitude of apparent energy expenditure that characteristically occurs at the peak of the hypoxic response. Lateral trunk curls, head tosses and rostral foreleg extensions are stereotypic motor acts that comprise the majority of fetal movements during the hypoxic response, but these patterns of movement are rare or absent during spontaneous fetal behavior (16). Moreover, these patterns of movement would effectively direct force away from the long axis of the fetus and result in pronounced changes in fetal posture. As with the quantitative changes in activity, these component responses were most evident on day 19 and least evident on day 21.

Coincident with the three-phase behavioral response, fetuses exposed to umbilical cord occlusion exhibited a monotonic decrease in fetal heart rate at all three gestational ages. No evidence of fetal tachycardia was obtained, in contrast to some earlier studies of fetal sheep maternal hypoxia (5,6), but consistent with the bradycardia reported for fetal sheep during partial umbilical cord compression (10). In the present study, bradycardia during hypoxic episodes was highly replicable between subjects: 31 of 32 fetuses exhibited heart rate deceleration within 15 sec. Fetuses exhibited more pronounced initial heart rate deceleration on day 21 than younger fetuses. Patterns of motor and cardiac response during acute hypoxic episodes therefore appear to be dissociated.

One explanation for the existence of a stereotypic and replicable fetal response to hypoxia is that it is a prenatal precursor to adaptive postnatal behavior and physiology. This seem improbable, however, because postnatal animals do not exhibit hyperactivity during hypoxia (6,7). The alternative to an interpretation of anticipatory function is that the hypoxic response is functional in utero. Behavioral and physiological processes that are functional only during early stages of development have been termed ontogenetic adaptations (8,12). The response of the rat fetuses to umbilical cord occlusion therefore may be an example of an ontogenetic adaptation restricted to the prenatal period.

Several facts support the interpretation of the fetal hypoxic response as an ontogenetic adaptation: (a) The form and intensity of the behavioral response at the peak of activity plausibly may exert force on a physical source of umbilical cord compression, thereby increasing the probability of removing the cause of hypoxia. We have observed that spontaneous fetal activity often results in distension of the wall of the uterus or alteration in fetal body orientation within the uterus, a fact which suggests that the vigorous motor activity associated with the hypoxic response is sufficiently energetic to exert considerable force against a mechanical source of umbilical cord compression. Indeed, the microvascular clamp used to induce hypoxia was occasionally dislodged as a result of fetal hyperactivity. (b) Increased activity during hypoxia would be counteradaptive if it did not contribute to restoration of umbilical circulation because it results in more rapid depletion of limited oxygen reserves. (c) Reduction in the intensity of the behavioral response to hypoxia near term may facilitate preparation for birth, which results in a sudden change of environment in which the term fetus must undergo a physiological and behavioral transition to an air-breathing neonate. (d) The hypoxic response of the rat fetus is unlikely to be a prenatal expression of postnatal behavior because neonatal rats exhibit the opposite pattern of response to acute hypoxia (7). For these reasons, the stereotypic behavioral response of the rat fetus to acute hypoxia is perhaps best viewed as an ontogenetic adaptation that originates, becomes functional, and disappears during the prenatal period (2,20).

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