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Variation in Motor Activity on Different Time Scales and Responsiveness to Oral Stimulation in the Rat Fetus

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ABSTRACT: *The near-term rat fetus exhibits brief oral grasp responses to discrete presentations of an artificial nipple. In the present experiment, an artificial nipple was presented to individual fetal subjects 10 times. Five of the presentations were timed to occur when spontaneous fetal motor activity was low and five while activity was high, as determined by the baseline activity for the individual fetus. The likelihood of responding to the artificial nipple was increased when the fetus was relatively inactive at the moment of stimulus presentation. Furthermore, stimulus presentations that resulted in oral grasping of the artificial nipple were associated with greater point-to-point variability (2-s intervals) in motor activity during the 30-s period preceding the presentation of the artificial nipple. This pattern of results indicates that the recent history of general motor activity as well as the level of activity at the instant of stimulus presentation may contribute to variation in responding to biologically relevant stimuli early in development.* © *1998 John Wiley & Sons, Inc.* Dev Psychobiol 33: 125–131, 1998

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Study of the rat fetus in utero provides a model system to examine behavioral and physiological responses to sensory stimulation in early development (Smotherman & Robinson, 1991, 1996). Because sensory stimuli are presented during the prenatal period, before the developing organism has experienced the stimuli in a functional context, the experimenter can examine the role of nascent central nervous system organization in the initiation and expression of motor patterns that will be necessary for the young organism's adaptation and survival after birth (Smotherman & Robinson, 1990).

It is well established that the rat fetus exhibits spontaneous motor activity before birth (Bekoff & Lau, 1980; Narayanan, Fox, & Hamburger, 1971; Robinson & Smotherman, 1987; Smotherman & Robinson, 1987a). This activity is evident by Day 16 of gestation (E16), reaches plateau by E19 and continues through term on E21 (Smotherman & Robinson, 1986). Fetal activity comprises movements of different parts of the body that can occur in isolation or in complex action patterns where several body parts are in motion at the same time. The bulk of fetal activity involves movements of the fore- and rearlimbs, with movements of the head and mouth occurring somewhat less frequently (Robinson & Smotherman, 1987). Fetal activity is not constant but fluctuates such that periods of

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relative activity and inactivity alternate, with the fluctuations occurring on a time scale of seconds (Smotherman, Robinson, & Robertson, 1988).

During the fluctuating stream of general motor activity, the rat fetus also can exhibit responsiveness to sensory stimulation. Different forms of sensory stimulation can evoke highly organized patterns of motor behavior. Infusion of stimuli into the oral cavity can trigger organized behavioral responses. For example, an intraoral infusion of milk (bovine light cream) can elicit a brief bout of mouthing, increase movements of the rear limbs, and result in the expression of a stretch response (Smotherman & Robinson, 1987b). Other chemosensory stimuli, including solutions of botanical extracts with strong olfactory properties such as mint or lemon, typically evoke aversive responses including facial wiping strokes (Smotherman & Robinson, 1987b). Tactile stimuli also can elicit organized motor responses from the fetal rat. For example, presentation of an artificial nipple (AN) sculpted from a soft vinyl material can elicit oral appetitive behavior that culminates in the expression of an oral grasp response. This oral grasp response involves directed movement of the head before the fetus closes its mouth around the tip of the nipple (Robinson et al., 1992). In general, responses to sensory stimulation involve elements of behavior that are present in spontaneous activity (Robinson & Smotherman, 1992a, 1992b).

Typically, presentation of stimuli such as milk, different botanical extracts, and the artificial nipple evoke organized behavioral responses from fetal subjects. However, sometimes the fetus fails to respond to these stimuli. Because the fetus is active in utero, and because complex responses to sensory stimulation may be assembled from elements of spontaneous activity, the recent history of spontaneous activity as well as its current level may influence responding to specific stimuli. Recent experiments by Reilly, Robertson, MacLennan, and Smotherman (1997) provide empirical evidence consistent with the hypothesis that the recent history of behavior is important. In those experiments, continuous exposure to an artificial nipple resulted in nonspecific behavioral activation, and increased variability during the minute preceding discrete presentation of the artificial nipple was associated with mouthing and oral grasping of the artificial nipple.

These results suggest that increased variability of overall activity may facilitate expression of well-defined action patterns. However, it is unclear how the level of activity at the moment of stimulus presentation and the recent history of activity jointly influence responsiveness to stimulation. In the present study, E21 fetal subjects were given a series of discrete presentations of an artificial nipple. Presentations of the AN were made at times of relatively high or low levels of fetal motor activity. Analyses focused on the relation between oral grasping and motor activity at the moment of stimulus presentation and during the preceding 30 s.

METHOD

Subjects

Subjects were the progeny of pregnant Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), produced by time-matings in the Laboratory of Perinatal Neuroethology at Binghamton University—SUNY. A total of 13 female rats provided 1 subject fetus each for testing in the experiment. Adult rats were housed in groups of 3 females and 1 male in plastic breeding cages measuring 36 × 47 × 20 cm during a 4-day breeding period. Vaginal smears were collected daily, with the 1st day of detectable sperm designated as embryonic Day 0 (E0; with birth occurring on E21.5). After removal of the breeding male, pregnant female rats were maintained under conditions of constant room temperature (22°C ± 1.0°C), on a 12-12:12 hr light:dark cycle (lights on at 0700/hr) until the day of fetal testing (E21). Food and water were available ad libitum. At all times, rats were maintained and treated in accordance with guidelines for animal care and use established by the National Institutes of Health (1986).

Preparation of the Pregnant Female and the Fetal Environment

On E21, each pregnant rat was prepared surgically for behavioral testing of a fetal subject. The rat was placed briefly under ether anesthesia and then received a 90 μl injection of 100% ethanol into the spinal column between the first and second lumbar vertebrae. This procedure blocks neural transmission within the spinal cord at the low thoracic level and eliminates sensation in the lower part of the body. The pregnant rat was placed in a holding device and immersed to chest depth in a temperature-regulated water bath containing a buffered isotonic saline solution that was maintained at 37.5°C. Throughout the period of fetal testing, the activity of the pregnant rat was monitored visually to ensure completeness of the spinal preparation.

Direct access to the subject fetus was made possible by externalizing the uterus from the abdomen into the saline bath through a low, midline laparotomy. The individual fetus selected as the experimental subject

was delivered from the uterus and surrounding membranes into the bath, taking care to preserve the umbilical attachment of the fetus to the placenta, which remained within the uterus. The condition of the fetus was monitored visually during the experiment. Only fetuses that remained pink and apparently well oxygenated were used as experimental subjects. After surgical preparation, a 20-min period elapsed to provide time for the pregnant rat and subject fetus to accommodate to the bath environment before the start of behavioral testing. After completion of testing, female and fetuses were sacrificed humanely. Additional details of these surgical procedures are provided in Smotherman and Robinson (1991).

Recording of Gross Motor Activity

The recording electrode was constructed from the shank of a 25-ga needle attached to a copper wire (diameter 0.15 mm). The electrode was implanted under the skin on the midline of the subject's back and connected to a custom-made, high-gain amplifier. The movement signal from the electrode was amplified (gain of 500) and low pass filtered (cut-off = 90 Hz). The filtered signal was passed to a contour follower integrator with a .25-s time constant (Friedlund, 1979). Fetal movement detected by the electrode was sampled at 15 Hz and digitized on-line throughout the experimental session to provide a measure of overall fetal motor activity (Reilly et al., 1997). The average length of the test session was 13.5 min ($\pm$ 0.5 min).

Presentation of the Artificial Nipple and Scoring of the Oral Grasp Response

The artificial nipple (AN) was fashioned from a block of soft vinyl material (Carolina Biological Supply Co., Burlington, NC), cut to 25 mm in length and tapered to a diameter of 1 mm at a rounded tip. The base of the artificial nipple was attached to the handle of a dental probe to facilitate manual presentation by the experimenter. Presentation involved the gentle placement of the AN in contact with the subject's mouth, without forcing the tip into the mouth (Robinson et al., 1992). Movement of the AN was limited to small adjustments to maintain contact between the AN and the perioral region of the subject fetus. Movement of the AN did not cause the fetus to be moved or create fluid currents.

Presentation of the artificial nipple can evoke an oral grasp response from the E21 fetal subject. During expression of an oral grasp response, the subject closes its mouth around the nipple and exerts negative pressure on the tip of the nipple. An attempt to remove the nipple from the mouth of the fetus is met with resistance (Robinson et al., 1992). An oral grasp of the AN was indicated by the experimenter using a handheld event recorder that marked the motor-activity time series. A video camera (Canon Super Macro AF 6–60 mm) positioned 65 cm directly above the fetal subject provided a video record (at 30 frames/s) of the experimental session. Reduced speed analysis of videotape records was performed at the conclusion of the experiment to confirm the timing and duration of individual oral grasp responses.

Experimental Design

A 3-min period at the beginning of each recording session served to establish a baseline level of motor activity for each subject. On-line analysis of the motor activity data signaled delivery of the AN when the subject's instantaneous motor activity was in the upper third (high condition) or lower third (low condition) of the baseline range for a period of 2 consecutive seconds. Individual fetal subjects were exposed to the AN 10 times with five of the exposures in the high condition and five of the exposures in the low condition. The order of presentation was dictated by the subject's motor activity. Each of the AN presentations lasted for a period of 15 s and was followed by an interstimulus interval of at least 45 s during which no AN presentation was made. Following each interstimulus interval, the subsequent AN presentation was made as soon as the instantaneous motor activity of the fetus again met the criterion for either the high or low condition.

Data Analysis

The data analysis had two objectives. The first objective was to determine whether the number and duration of oral grasp responses differed according to whether the AN was presented when instantaneous activity was high (high condition) or low (low condition).

The second objective of the data analysis was to compare the characteristics of motor activity over an extended time period preceding presentation of the AN. Specifically, the level and variability in overall motor activity during the 30-s preceding AN presentations were compared for presentations that did or did not elicit an oral grasp response. To accomplish the second objective, the level and variability in motor activity were calculated for each fetus for the 30-s preceding each AN presentation. First, a 5-s moving minimum was subtracted from the unprocessed time series data to remove baseline instability. Next, the resulting

time series was integrated in nonoverlapping 2-s windows (Reilly et al., 1997; Robertson et al., 1996). The average of the fifteen 2-s windows preceding each AN presentation was used as a measure of the level of overall motor activity. The average of the absolute values of the point-to-point differences (2-s windows) in the motor-activity time series provided a measure of the variability in overall activity.

Results

Distribution of High and Low Conditions. The distribution of high and low conditions was examined to determine whether they occurred equally often in the first and second halves of the experimental session. The mean number of high presentations in the first half of the experimental session was 2.54, which does not differ from a random distribution of the conditions, $t(12) = .11$, $p = .91$.

Number of Oral Grasp Responses. A paired *t* test was used to compare the number of oral grasp responses to AN presentations in high and low conditions of instantaneous motor activity. This analysis indicated the significant effect of conditions, $t(12) = 2.6$, $p < .05$ (Figure 1). Fetal subjects expressed significantly more oral grasp responses to AN presentations in the low condition compared to the number of oral grasp responses expressed in the high condition.

Duration of the Oral Grasp Responses. The durations of individual grasp responses for each fetus were averaged separately for the high and low conditions. A paired *t* test was used to compare the average duration of oral grasp responses to AN presentations in the high and low conditions of instantaneous motor activity. This analysis indicated the significant effect of conditions, $t(12) = 3.0$, $p < .02$ (Figure 2). Fetal subjects showed significantly longer oral grasp responses in the low condition compared to the duration of the oral grasp responses in the high condition.

Level of Preceding Motor Activity. A two-factor (Conditions: High, Low × Oral Grasp: yes, no) repeated measures ANOVA was used to compare the level of motor activity during the 30-s period preceding AN presentations. For this analysis, levels of preceding motor activity for each fetus were averaged separately within high and low conditions for presentations that did or did not result in oral grasping of the AN. This analysis revealed a significant main effect of conditions, $F(1, 24) = 28.8$, $p < .001$ (Figure 3). The main effect of oral grasp, $F(1, 24) = 2.7$, $p > .10$, and the interaction of conditions and oral grasp, $F(1, 24) = 2.5$, $p > .10$, were not significant. These results indicate that the level of motor activity during the 30-s period preceding AN presentations was greater in the high condition than in the low condition, as expected. However, the level of preceding motor activity did not differ for AN presentations which elicited an oral grasp response and those

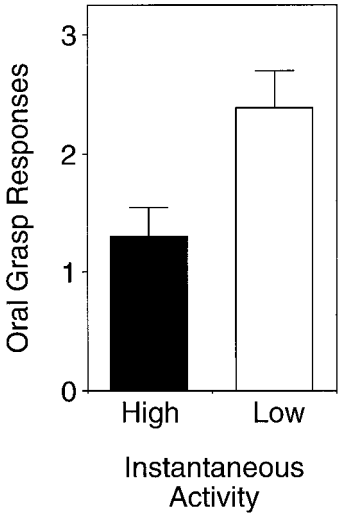

FIGURE 1 Number of oral grasp responses for fetal subjects presented with the artificial nipple (AN) under high or low conditions of instantaneous motor activity. Bars represent means, vertical lines depict *SEM*.

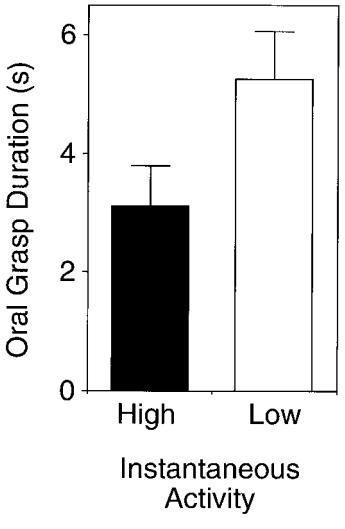

FIGURE 2 Duration of oral grasp responses (mean ± *SEM*) for subjects presented with the AN under high or low conditions of instantaneous motor activity.

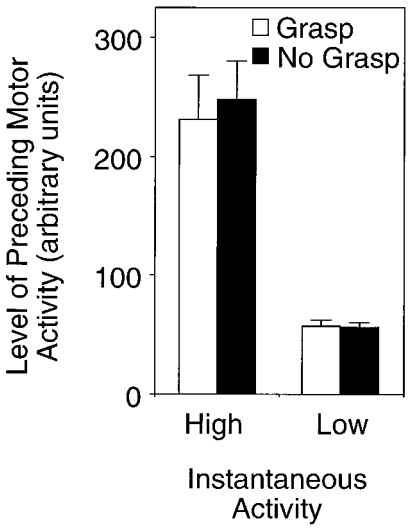

FIGURE 3 Level of preceding motor activity (mean ± *SEM*) for fetal subjects presented with the AN under high or low conditions of instantaneous motor activity. Data are presented separately for presentations that did or did not result in an oral grasp response.

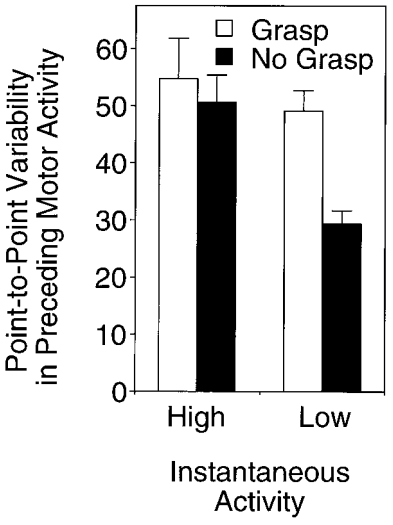

FIGURE 4 Point-to-point variability (mean ± *SEM*) in preceding motor activity for fetal subjects presented with the AN under high or low conditions of instantaneous motor activity. Data are presented separately for presentations that did or did not result in an oral grasp response.

that failed to elicit oral grasping, regardless of condition.

Variability in Preceding Motor Activity. A two-factor (Conditions: High, Low × Oral Grasp: yes, no) repeated measures ANOVA was used to compare the point-to-point variability in motor activity during the 30-s period preceding AN presentations. For this analysis, point-to-point variability of preceding motor activity for each fetus was averaged separately within high and low conditions for presentations that did or did not result in oral grasping of the AN. This analysis revealed an interaction between condition and oral grasp, $F(1, 24) = 6.9$, $p < .02$ (Figure 4). There was no difference in the point-to-point variability of preceding motor activity for presentations that did or did not elicit an oral grasp response within the high condition, $t(12) = .46$, $p > .65$. In contrast, in the low condition, the point-to-point variability of preceding motor activity was greater for AN presentations that did elicit an oral grasp response compared to presentations that did not elicit an oral grasp response, $t(12) = 5.4$, $p < .0002$.

DISCUSSION

Previous studies have established that the near-term fetal rat exhibits brief oral grasp responses to discrete presentations of an artificial nipple (Robinson et al., 1992). The present experiment demonstrates that the likelihood of responding to the artificial nipple is increased when the fetus is relatively inactive at the instant of stimulus presentation. Furthermore, stimulus presentations that result in an oral grasp response are associated with greater variability in motor activity during the 30-s period preceding the presentation. Therefore, variation in spontaneous motor activity on two different time scales jointly influences the responsiveness of the fetus to this model of a biologically relevant stimulus.

Stimulus presentations that resulted in an oral grasp response were associated with greater variability in motor activity during the preceding 30 s period and a low level of activity at the time of stimulus presentation. Variability as defined in this study refers to alternations in activity level across consecutive 2-s intervals for an extended period of time. That is, increased point-to-point variability implies that higher levels of spontaneous activity occur in repeated brief intervals that are interspersed with periods of lower activity. Grasping of the artificial nipple by the fetal rat involves components of spontaneous activity including movements of the head, oral appetitive behavior such as mouthing and licking, capture of the nipple, biting, and compression pressure on the tip of the nipple (Robinson et al., 1992). Periods of higher levels of spontaneous activity may result in the coactivation of

these component behaviors and thereby facilitate the assembly of oral grasping, a coordinated motor response evoked by this biologically significant form of stimulation.

The clustering of spontaneous activity also means that there are moments of relatively low activity interspersed with periods of higher activity. The present experiment demonstrates that the likelihood of responding to the AN is increased when the fetus is relatively inactive at the moment of stimulus presentation. It is during these moments of relative quiescence that the fetus may be open to the influence of stimulation from the environment so that specific features of a stimulus can influence the particular patterns of behavior that are expressed by the fetus.

Previous studies have established that the near-term fetal rat exhibits brief oral grasp responses to discrete presentations of an artificial nipple (Reilly et al., 1997; Robinson et al., 1992). The present study demonstrates that expression of an oral grasp response is increased when there is a period of time preceding the artificial nipple presentation where the motor system is showing a high level of variability and when the subject is relatively quiescent at the instant of stimulus presentation. Understanding the contribution of variability in motor activity to the expression of coordinated action patterns in early development is a relatively new and potentially important area for developmental theory (Robertson, 1988; Robertson & Bacher, 1995). The results of the present study provide empirical support for a broadly dynamic approach to understanding behavioral organization. That is, the expression of particular forms of behavior at any instant of time depends jointly on the stimulus conditions, the behavior of the organism at that moment, and the recent history of behavior leading to that moment.

However, the results of the present study take us beyond general statements about the flow of behavioral organization on multiple time scales and suggest some specific ways in which the features of real behavior might interact to alter the likelihood of adaptive responding to the environment in a particular model system. Specifically, during bouts of spontaneous movement, components of complex behavior patterns may be activated, while during intervening moments of relative quiescence, sensory input may gain control. The result of this activation and control is the assembly of an adaptive response to a biologically relevant stimulus. Furthermore, characterizing the role of variability in motor behavior may suggest ways to promote the precocial expression of adaptive behaviors at earlier developmental stages or may indicate strategies for intervention when atypical patterns of early development are evident.

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