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Accessibility of the Rat Fetus for Psychobiological Investigation

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Behavioral development does not start at birth. Any woman who has been pregnant is aware that the fetus is active in utero. Until recently, in fact, maternal reports of fetal movement constituted the principal means of access to the fetus for the study of behavior. Innovative technologies, such as external fetal monitoring and real-time ultrasound, envisioned for monitoring fetal well-being and managing high-risk pregnancies, have recently been applied to the description of fetal movement (deVries, Visser, & Prechtel, 1982, 1985) and sensory development (Birnholz, 1984; Birnholz & Benacerraf, 1983). In the last five years, these methods have begun to offer an alternative to indirect inference about the behavioral abilities of the human fetus. The advent of an electronic window on fetal development is challenging long-held notions of the fetus as an intrauterine passenger. Clearly, the fetus is responsive and exhibits patterned motor activity. Understanding the relationship between prenatal activity and postnatal behavior, however, requires the application of detailed quantitative and experimental methods.

Aside from practical concerns, such as improving clinical care of premature infants, there are fundamental issues of theoretical importance that will benefit from study of the fetus. Is the fetus's behavior shaped by adaptation to its intrauterine environment or by the needs of preparation for future environments? On the one hand, it is apparent that the fetus is an active organism that responds to changes in the intrauterine environment. Some elements of the fetal repertoire, therefore, may serve as ontogenetic adaptations, improving the fit between the fetus and its surroundings. On the other hand, at no point during development does a young mammal experience a more radical change of environment than at the time of birth. The behavioral competence of the newborn argues that the neonate is prepared immediately after birth to interact with its postnatal environment. Preparation for postnatal life implies the existence of behavioral continuity: The roots of neonatal competence lie in the prenatal period. The issues of ontogenetic adaptation, preparedness, and continuity are especially relevant for the fetal period, but in broader perspective apply to the problem of behavioral development over the entire lifespan. Because the distinction between these issues is most clear at this point in development, understanding of prenatal behavior as well as processes of behavioral ontogeny in general should result

from extension of a perspective of behavioral epigenesis to encompass events that precede birth.

In order to build an empirical foundation for addressing these issues, we have employed a comparative approach to the study of fetal behavior. Experimentation with human fetuses is an ethically sensitive and emotionally charged issue. Moreover, guidelines for the study of human fetuses place limitations on what can be learned from direct study of human fetuses. Therefore, comparative study of nonhuman animal fetuses is imperative. In this chapter, we describe methods currently employed in our laboratory to study the behavior of the rat fetus. Direct fetal observation necessitates (1) humanely desensitizing the pregnant rat without exposing fetuses to general anesthesia, (2) surgically preparing the uterus and fetuses to bring them into direct view, and (3) maintaining the viability of subject fetuses throughout the period of observation. In addition, behavioral study is greatly aided by (4) methods of manipulating the local physical and sensory environment of the fetus. The technical ability to view the fetus is incomplete, however, without (5) a correspondingly precise vocabulary of fetal movement and rigorous methods for describing and quantifying fetal behavior. These surgical and analytical tools make the rat fetus accessible for behavioral investigation.

PREPARATION OF THE PREGNANT RAT

The principal difficulty with devising ways to directly observe live fetuses is satisfying the conflicting needs of maintaining a normal physiological environment for the fetus while minimizing discomfort and trauma for the mother. The first attempts to view animal fetuses involved study of dying fetuses (Preyer, 1885). At the other extreme, researchers of the 1920s and 1930s often studied fetuses while mothers were under the effects of complete general anesthesia (Swenson, 1926). Under these conditions, fetuses show little behavior. Excluding classical methods, such as decerebration produced by ligation of the carotid arteries (Angulo y Gonzalez, 1932), two techniques were generally available prior to the 1980s that permitted direct observation of fetuses while circumventing the effects of general anesthesia. Physical transection of the spinal cord was developed early in fetal research (Windle & Becker, 1940) and has been employed in recent studies of fetal motor activity (Narayanan, Fox, & Hamburger, 1971; Kirby, 1979; Kodama & Sekiguchi, 1984). Physical transection of the spinal cord is effective in producing posterior paralysis and eliminating sensation in the lower limbs and abdomen. A number of variant techniques are available for transecting the spinal cord, but most involve some degree of surgical incision through the skin, dorsal musculature, and vertebral column to gain access to the spinal cord.

An alternative procedure developed in the 1960s—chemomyelotomy—achieves the same end as spinal transection with a simple intraspinal injection of ethanol (Basmajian & Ranney, 1961). Chemomyelotomy results in complete interruption of nervous transmission within the spinal cord at the site of injection and paralysis of the hindquarters and lower abdomen. This procedure has also been used effectively in the study of fetal behavior (Narayanan, Narayanan, & Browne, 1982). We currently use the following procedure to perform a chemomyelotomy on pregnant rats (Smotherman, Richards, & Robinson, 1984). The rat is placed in a jar suffused with anesthesia-grade ethyl ether until fully anesthetized. Ether is used for initial anesthesia because it

takes effect quickly and is eliminated from the body rapidly after the rat is exposed to room air. An alternative short-acting anesthetic, such as halothane, may be preferred for species that are especially sensitive to ether.

Once anesthetized, the rat is removed from the jar and placed in a prone position on a surgical board. A small area of fur overlying the midback is removed with clippers. To facilitate visual guidance of the injection, a small midline incision (5–10 mm) is made in the skin, not extending into the musculature. The injection of 100% ethanol (100 μ l for 300–400 g subjects, delivered at room temperature) is accomplished by inserting a 25-gauge needle, oriented perpendicular and dorsal to the spine, into the space between the first and second lumbar vertebrae. This site is identified by counting dorsal spinal processes posterior to the last pair of ribs. The tip of the needle is then directed caudally within the vertebral foramen and the ethanol injected in a 1–2 s pulse. The head and thorax of the rat should be slightly elevated immediately after injection to prevent rostral flow of ethanol within the vertebral canal. After the injection, prepared rats typically exhibit hyperventilation, extension of both hindlimbs, and nipple erection. When learning this technique, investigators may find it necessary to apply a nose cone with ether until the injection is delivered. But after training, the entire procedure requires only 1–2 min after removal of the rat from the ether jar, and no supplemental anesthetic is required.

Spinal transection and chemomyelotomy are outwardly similar in their effects on the pregnant rat. Both result in irreversible spinal blockade that permits surgical externalization of the uterus without the use of general maternal anesthesia. Under either of these preparations, fetuses can be observed without evidence of physiological impairment for periods exceeding one hour. However, spinal transection and chemomyelotomy are not equivalent in their effects on spontaneous fetal behavior. With mothers prepared by spinal transection, fetuses exhibit slightly more motor activity than fetuses from mothers prepared by chemomyelotomy (Smotherman, Richards, & Robinson, 1984). While differences in overall activity do not pose great problems for comparison between procedures, of greater concern are independent and unpredictable effects of the two procedures on specific patterns of fetal behavior. Differential behavioral effects, where present, seem to be most pronounced in older fetuses (day 20).

We believe there are several objective reasons for preferring chemomyelotomy over spinal transection as a method of preparing pregnant rats for fetal study: (1) Chemomyelotomy is simple in application and is readily taught to investigators or students unfamiliar with the procedure. (2) The effects of chemomyelotomy are consistent between subjects, providing high-quality fetal preparations with low rates of accidental mortality (about 1%). (3) Injection of ethanol into the spinal cord, in contrast to the surgical excision of dorsal musculature and vertebral segments employed in spinal transection, results in very little local trauma to the prepared female. This fact alone may contribute to the differential effects these two procedures have on fetal behavior. Therefore, while we would not wish to ignore the utility of spinal transection or eliminate its use in specific situations, we generally prefer the use of chemomyelotomy in behavioral applications, if for no other reason than to facilitate comparison of research findings obtained in different laboratories.

Spinal transection and chemomyelotomy are irreversible procedures; pregnant rats must be sacrificed after fetal observation. However, certain research questions,

especially those concerned with issues of continuity between the prenatal and postnatal period, will benefit from an alternative preparation that is reversible. Lidocaine spinal anesthesia provides a means for direct observation of fetuses without terminating gestation (Smotherman, Robinson, & Miller, 1986). This procedure involves the same anesthesia and injection protocol as is used in performing a chemomyelotomy. A lidocaine solution containing epinephrine (2% lidocaine + 0.001% epinephrine) is injected into the spinal cord at the same level as chemomyelotomy (L1–L2). Intraspinal injection of 100 μ l of the lidocaine solution is effective in satisfying the principal objectives of a reversible procedure: complete abdominal and hindlimb paralysis, consistently long periods of spinal anesthesia (in excess of 50 min), and complete recovery after anesthesia. After recovery, when fetuses are observed through the transparent wall of the uterus (see below), mothers can continue the pregnancy, deliver pups at term, and exhibit apparently normal maternal behavior. The potential for this reversible maternal preparation to facilitate longitudinal comparison of fetal and pup behavior is obvious.

Unlike spinal transection, there is no evidence that reversible lidocaine spinal anesthesia differs in its effects on fetal behavior from chemomyelotomy. Similar levels of overall fetal activity, synchronous movements, repertoire diversity, and patterning of individual fetal movements have been reported in the two preparations, suggesting that they are true alternatives (Smotherman, Robinson, & Miller, 1986). Lidocaine anesthesia should be preferred in experiments calling for longitudinal analysis of an individual subject through advancing stages of gestation and extending into the postnatal period. Chemomyelotomy offers the advantage of extended periods of observation, permitting manipulation of the intrauterine environment (Smotherman & Robinson, 1988a) and temporal analyses of fetal behavior (Smotherman, Robinson, & Robertson, 1988; Robinson & Smotherman, 1988). Both procedures result in prepared females that remain generally quiet during observation sessions and show no outward signs of discomfort. We view the development of these maternal preparations as a significant advance over the methods commonly used and ultimately discontinued during the early years (1920–1940) of fetal research.

PREPARATION OF SUBJECT FETUSES

Once the pregnant rat has been prepared with some form of spinal blockade, it is placed in a Plexiglas holding apparatus that elevates the head and body 45° above the horizontal (Smotherman, Richards, & Robinson, 1984). The rat is secured in the apparatus with a velcro jacket. The lower abdomen is shaved, and a low midventral incision (about 3 cm in length) is performed. The female and apparatus are then placed in a buffered isotonic saline solution (Locke's solution [Galigher & Kozloff, 1971]) with temperature regulated at 37.5°C \pm 0.5°C. Water depth is adjusted to about the tip of the sternum, ensuring that the head is above and the incision below the water surface. Both horns of the uterus, and the constituent fetuses, are gently externalized through the abdominal incision into the saline bath. This manipulation is accomplished without placing strain on points of uterine attachment at the ovarian ends or at the cervix. The mother and fetuses remain undisturbed in this environment until a period of 15–20 min after the termination of general anesthesia has elapsed. This delay is fully adequate to ensure no residual effects of ether anesthesia on the

mother or, more critically, on the behavior of fetuses (Kirby, 1979; Smotherman, Richards, & Robinson, 1984). The female should be monitored during this period to prevent uterine torsion from occluding blood flow within the uterus.

The fetus can be thought of as occupying a physical environment that is separated from the external world by successive envelopes or barriers. These envelopes, progressing from the outermost to the innermost, include the maternal abdomen, the wall of the uterus, the extraembryonic membranes (amnion and chorion), and amniotic fluid. Investigation of fetal behavior is greatly facilitated by stripping away successive envelopes, creating a series of different physical environments in which the spontaneous or stimulus-evoked behavior of fetuses can be observed. Each fetal environment offers different advantages and drawbacks for experimental study of the fetus.

Immersion of the pregnant rat and externalization of the uterus into the saline bath effectively strip away the outermost envelope surrounding the fetus. At this level of preparation individual fetuses can be viewed through the semitransparent wall of the uterus. Because the uterus is undisturbed throughout observation, observation of rat fetuses *In Utero* (as we have referred to this environment) most closely approximates normal intrauterine conditions during gestation. The wall of the uterus becomes thinner and more transparent as gestation proceeds. For this reason, observation *In Utero* is impractical earlier than about day 16 of gestation.

A second level of fetal preparation involves externalization of a single conceptus into the saline bath (*In Amnion*). The conceptus, which comprises a fetus, amniotic fluid, and surrounding membranes, is delivered through a small (10–20 mm) transverse incision in the uterine wall. Care should be taken in locating this incision, as it can influence the physiological condition of the fetus and the length of time that it can be observed. The incision should be placed on the side of the uterus facing away from uterine blood vessels and sites of placental attachment. If a fetus in a terminal position (closest to the ovary) is selected for study, a standard protocol we have often adopted in our research, a single incision can be made between the subject and other fetuses in the horn. An incision in this location minimizes the potential for adjacent fetuses to press against the placenta of the subject fetus and induce placental separation from the uterus. If a subject is selected from a miduterine position, a second incision isolating the subject from ovarian and caudal neighbors can be made. A second incision is especially useful when many fetuses occur in the same uterine horn or late in gestation, when intrauterine crowding is greatest. After externalization, the fetus *In Amnion* can be observed in considerable detail through the transparent amniotic and chorionic membranes. Throughout observation *In Amnion* the fetus remains attached to its placenta, which is not delivered through the incision and remains within the uterus. Because the extraembryonic membranes become very fragile and are prone to rupture near term, observation of rat fetuses *In Amnion* on day 21 is technically difficult.

The ultimate envelope around the fetus—the extraembryonic membranes—can be removed in a third-level preparation that provides the clearest view of the fetus and unobstructed access for experimental manipulation (*Ex Utero*). After an incision is made in the uterine wall (as in the *In Amnion* preparation), a small cut is made in the chorion near the head of the fetus. The incision in the membrane is made before delivery of the conceptus from the uterus. In this way the fetus is gently delivered

headfirst into the bath while the chorion, amnion, and placenta are protected within the uterus. Because the fetus remains attached to the placenta by means of the umbilical cord, delivery into the saline bath does not impair fetal viability. Twisting of the umbilical cord at this point, or permitting a neighboring fetus to press against the cord, can lead to occlusion of umbilical circulation. One method of preventing this is to make a secondary incision in the uterus caudal to the neighboring fetus. The cord should be monitored throughout observation. We have routinely observed fetuses *Ex Utero* on days 17–21 of gestation for periods in excess of 30 min without evidence of fetal or placental compromise (Smotherman & Robinson, 1986a; Smotherman, Robinson, & Robertson, 1988).

We believe there is no “best” preparation in which to study fetal behavior. Each of the three fetal environments described above offers unique advantages and drawbacks for particular experimental needs (Smotherman & Robinson, 1988b). Fetuses observed *In Utero* remain in close proximity to contiguous siblings, permitting direct observation of prenatal behavioral interactions. If the pregnant rat is prepared by the reversible spinal anesthetic method, fetuses *In Utero* can be replaced within the maternal abdomen, the abdominal incision closed with sutures, and the litter allowed to continue gestation until normal parturition. The chemical constituents of the *In Uterus* or *In Amnion* environments can be altered experimentally by injecting various solutions into the amniotic fluid. Chemical manipulation of the amniotic fluid tends to persist in the fetal environment and thus provides a useful tool for investigating mid- to long-term effects of different chemical cues on spontaneous fetal behavior (Smotherman & Robinson, 1985), prenatal learning (Stickrod, Kimble, & Smotherman, 1982; Smotherman, 1982a, 1982b; Smotherman & Robinson, 1985), and morphological development (Moessinger, 1983). Short-term responsiveness to chemical stimuli is most conveniently studied with the fetus *Ex Utero*, where it is available for physical or surgical manipulation. The clearer view of the fetus afforded by observation *Ex Utero* also facilitates creation of real-time videotape or film records of fetal behavior. Playback of recorded behavior at reduced speed or frame by frame is necessary for close inspection and analysis of rapid, often subtle motor performances by the fetus (Bekoff & Lau, 1980).

In addition to serving particular experimental designs, observation of spontaneous fetal behavior in these three preparations has provided evidence that fetuses are responsive to changes in their physical environment. Especially late during gestation, when fetal body size and diminished amniotic fluid volume exacerbate physical crowding within the uterus, the spontaneous behavior of fetuses observed *In Utero* and *Ex Utero* is different in many respects (Smotherman & Robinson, 1986a). In view of this finding, one may wonder whether undisturbed fetuses that remain within the maternal abdomen, an environment that preserves all four envelopes surrounding the fetus, also exhibit different behavior. One approach to addressing this question is to use a minimally invasive optical device to visualize fetuses within the uterus inside the maternal abdomen (*In Situ*).

The endoscope we have employed to visualize rat fetuses *In Situ* is the same instrument widely used in arthroscopic surgery (Smotherman & Robinson, 1986b). It consists of a slender telescopic tube, a light source and fiber-optic cable, and a protective external sheath (Karl Storz Endoscopy-America). When connected to a microvideo camera, a wide-angle image can be displayed on a video monitor or

recorded with standard equipment. The endoscope's small diameter (5 mm) permits it to be inserted through a very small incision in the pregnant rat's abdomen. The resultant views of the fetus vary with fetal size (and therefore gestational age) and the position of the abdominal incision. Endoscopic images are never as clear as afforded by direct observation of fetuses within an externalized uterus, but they are sufficient to measure rates of overall fetal activity and document gross patterns of motor behavior. In general, the repertoire and levels of activity exhibited by fetuses observed endoscopically *In Situ* are comparable to those expressed by fetuses observed *In Utero*.

Because each of the four environments in which fetuses may be observed (*In Situ*, *In Utero*, *In Amnion*, and *Ex Utero*) offer unique advantages for particular experimental needs, all are appropriate for the study of fetal behavior. However, a trade-off exists between the similarity of a particular observation environment to the conditions that exist during undisturbed pregnancy and the degree of control over stimulus conditions afforded the experimenter. In moving from endoscopic visualization *In Situ* to direct observation *Ex Utero*, fetuses experience less physical restraint. On the other hand, controlled presentation of chemical or tactile stimuli and examination of detailed motor responses is greatly facilitated by stripping away successive envelopes that surround the fetus. We have found that rather than limiting investigation, study of fetuses in different environments actually offers advantage in terms of generating hypotheses about normal behavioral development.

To illustrate, consider the different lines of research that originally led to independent investigation of behavioral and cardiac response to hypoxia produced by occlusion of the umbilical cord, chemosensory development and responsiveness of fetuses to chemical stimuli infused into the mouth, and the ability of fetuses to form conditioned associations *in utero*. By necessity, each of these areas of inquiry required study of fetuses in environments that permitted manipulation of the fetus and/or its sensory environment (*In Amnion* or *Ex Utero*). Briefly, these experiments led to discoveries that (1) umbilical occlusion results in behavioral hyperactivity and expression of stereotypic action patterns (Smotherman & Robinson, 1987b). (2) rat fetuses express different patterns of behavioral activation to some chemical stimuli (such as lemon or milk), but fail to respond at all to other stimuli (such as sucrose) (Smotherman & Robinson, 1988a), and (3) fetuses are capable of forming associations between neutral chemical stimuli paired with aversive events (such as the illness reaction produced by *i.p.* injection of lithium chloride) (Smotherman & Robinson, 1985). Only when this information became available to us was a scenario suggested in which fetal learning could occur under naturalistic conditions.

Olfactory stimuli can be transmitted to the fetus during normal pregnancy by transplacental transfer into the amniotic fluid (Hepper, 1987) or more directly by exposure of olfactory receptors to blood-borne odorants (Maruniak, Silver, & Moulton, 1983). Accidental occlusion of the umbilical cord, leading to transient episodes of fetal hypoxia, is a relatively common event even in unremarkable pregnancies (Mann, 1986). Chance association of exposure to olfactory cues *in utero* with a transient hypoxic episode could confer behavioral significance to these odors. We have recently begun to evaluate this naturalistic hypothesis in an experimental paradigm by pairing intraoral infusions of sucrose with a brief period of umbilical cord

occlusion. Findings to date indicate that fetal conditioning does occur under these circumstances.

MANIPULATION OF FETUSES AND THEIR SENSORY ENVIRONMENT

In some respects, the fetus is a fragile organism. At term, only a small portion of the skeleton is ossified, and tissues offer little resistance to external force. While this fact underscores the need to manipulate fetuses carefully, it also can be made to serve the aims of the experimenter. Implantation of intraoral cannulas, installation of subcutaneous cardiac leads, and transection of the central nervous system can be accomplished with simple, rapid procedures that result in minimal trauma. Fetuses with cannulas or neural transections remain healthy and active and provide nearly ideal subjects for the study of early sensory, neural, and motor development.

Prior to 1980, much of what was widely believed about the sensory abilities of fetuses was based on histological examination of aborted fetuses (Humphrey, 1953). Direct behavioral assessment of fetal sensation was limited to experiments with a single sensory modality: touch. Evoking fetal movements by tactile stimulation is an experimental technique that was widely used in fetal reflexology during the 1920s and 1930s, and which has changed little in the past 50 years (Angulo y Gonzalez, 1932; Narayanan, Fox, & Hamburger, 1971). Punctate stimuli are delivered with a hand-held filament or needle; stimulus intensity can be quantified by varying the flexibility of the filament, and thus the force applied, with a pressure aesthesiometer consisting of von Frey filaments of different diameter (Stoelting Co.). Punctate stimulation has been used to describe developmental changes in simple motor reflexes and to chart body areas of the fetus that exhibit different thresholds of tactile sensitivity.

An alternative form of tactile stimulation involves repeated stroking of the fetus with a soft brush (Smotherman & Robinson, 1988a). Because stroking is administered to an area rather than a point, it is less suitable for detailed mapping of tactile sensitivity over the surface of the fetus. Stroking can be effective, however, in producing distinct effects on behavior, such as manipulation of overall levels of fetal activity. Tactile stimulation, whether by punctate contact or stroking, can be administered in a general way through the amniotic sac, the wall of the uterus, or even the maternal abdomen. However, the uterus, extraembryonic membranes, and amniotic fluid cushion the fetus, attenuate tactile stimuli, and yield results that suggest elevated tactile thresholds. Because the efficiency of transmission of mechanical force through these envelopes varies during gestation, controlled application of tactile stimuli requires that the fetus be removed from the amniotic environment and tested *Ex Utero*.

It is perhaps not surprising that fetuses are sensitive to touch. During early postnatal life, however, pups also depend on other forms of sensory stimulation to interact with their environment. Pups utilize olfaction and gustation to locate and suckle from the nipple (Blass & Teicher, 1980), olfaction to recognize the mother (Leon, 1974) and discriminate kin from nonkin (Hepper, 1986), and vestibular and thermal sensitivity to maintain a huddle and regulate body temperature (Alberts & Cramer, 1988). Recent indirect evidence has suggested that rats possess a functional chemical sense before birth (Pedersen, Stewart, Greer, & Shepherd, 1983; Pedersen & Blass, 1982; Smotherman, 1982a; Stickrod, Kimble, & Smotherman, 1982). This

conclusion is confirmed by direct behavioral assessment of fetal responsiveness to chemical stimuli (Smotherman & Robinson, 1985; 1988a), findings that have suggested a possible role for fetal olfaction in the development of chemical recognition (Hepper, 1987). Several techniques now exist for presenting chemical stimuli to the fetus in utero that allow for progressively greater control over the timing and intensity of stimulus exposure.

The most indirect method of exposing fetuses to chemical stimuli is perhaps the most relevant for fetal development. Specific substances in the diet of the pregnant rat can be transported by maternal circulation and diffuse across the placenta to the fetus. For example, garlic contains a sulfur-based compound (allyl sulfide) that is readily transmitted across the placenta. Pregnant rats that ingest garlic in their diet bear offspring that behave differently in the presence of garlic odor (Hepper, 1988). The effect of prenatal exposure may be due either to diffusion of allyl sulfide into the amniotic fluid, which is consumed by rat fetuses (Smotherman & Robinson 1988b), or to direct access of chemical receptors to blood-borne odors (Maruniak, Silver, & Moulton, 1983). While manipulation of maternal diet most closely approximates the conditions under which fetuses are normally exposed to external olfactorants, it does not permit measurement of fetal response at the time of exposure, assessment of age-related changes in chemosensation, or control over the moment of stimulus presentation or the intensity of stimulation.

Results paralleling the manipulation of maternal diet can be obtained by injecting chemical cues into the amniotic fluid that surrounds the fetus (Blass & Pedersen, 1980; Stickrod, 1981). Intra-amniotic injection is performed with the pregnant rat under general anesthesia. The uterus is externalized through a midline laparotomy and permitted to rest on the abdomen of the anesthetized, supine female. Chemical cues are introduced into the amniotic fluid of individual fetuses by injection of a small volume (20 μ l) of the test solution in an isotonic saline carrier. Solutions should be injected at the body temperature of the fetus. Use of a very fine needle (30 gauge) minimizes loss of amniotic fluid during injection. Further loss of amniotic fluid can be prevented by coating the site of injection with petroleum jelly or inserting the needle through the placenta. After injection, rotation of the conceptus within the uterus offsets the sites of injection through the uterine wall and the extraembryonic membranes and seals the amniotic sac from loss of fluid. After injection, the uterus is rinsed with isotonic saline and gently replaced within the maternal abdomen. The abdominal incision is closed with sutures and/or stainless steel wound clips and swabbed with a betadine solution. Mothers recover quickly from surgery and exhibit no adverse effects.

Fetuses exposed to chemical stimuli by intra-amniotic injection are not harmed and can be allowed to complete normal gestation. By exposing the fetus to a chemical stimulus and replacing it within the maternal abdomen, the chemosensory experience of the fetus can be manipulated several days before testing. Alternatively, intra-amniotic injection can be used to manipulate the local environment of the fetus minutes or seconds before testing when the female is immersed in the water bath. In this case, testing can take place with the fetus In Utero or In Amnion, although the latter preparation probably increases the rate of diffusion of the chemical cue from the amniotic fluid. Intraamniotic injection has been performed with rat fetuses between

days 17 and 21 of gestation. Comparison of fetuses manipulated by this procedure at different ages should take into account differences in stimulus concentration brought about by changes in amniotic fluid volume that normally occur during gestation (Smotherman & Robinson, 1988b).

Greater control over stimulus exposure (timing and concentration) is provided by direct infusion of test solutions into the mouth of the fetus (Figure 9.1). Intraoral

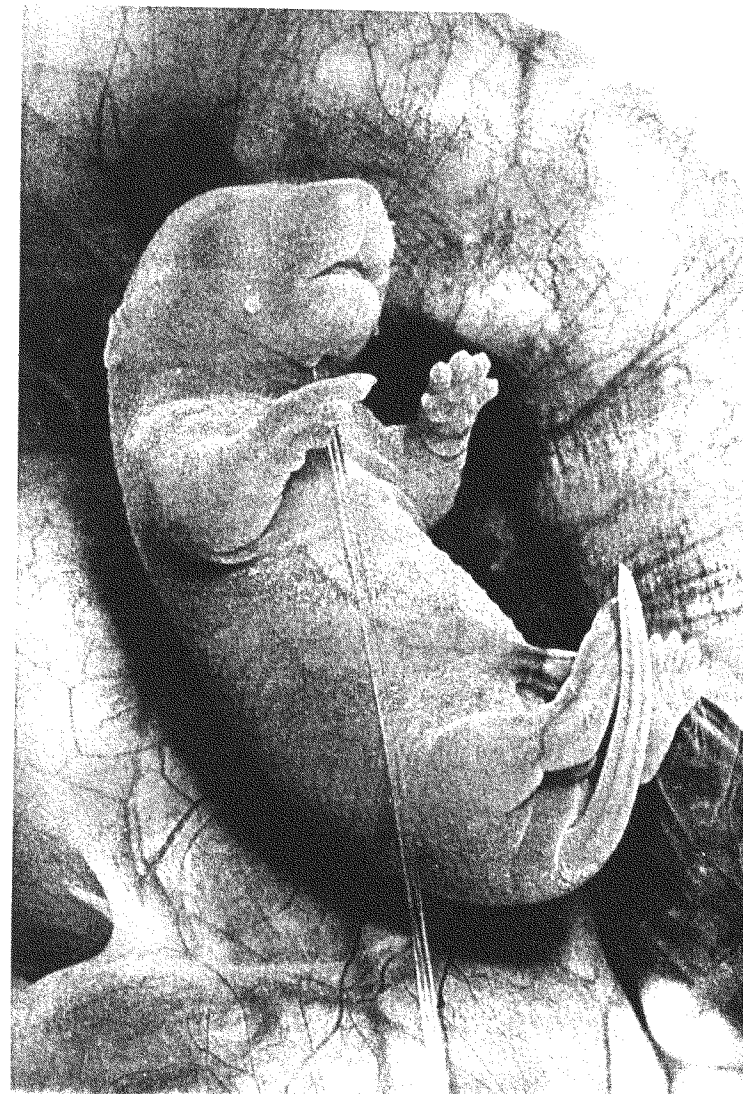


Figure 9.1. Rat fetus prepared for observation Ex Utero on day 20 of gestation. The fetus has been fitted with a cannula to permit intraoral infusion of a test solution.

presentation is made possible through the use of a cannula, adapted from procedures developed for rat pups (Hall & Rosenblatt, 1977; see also Phifer, chap. 13 this volume). During insertion of the wire involved in the installation of the cannula, care must be exercised to maintain the fetus under the surface of the water, to avoid crimping or pulling the umbilical cord, and to provide enough tubing so as not to restrict fetal movement. The cannula permits precise infusion of a test solution to the fetus without handling or otherwise interrupting ongoing fetal activity. We typically employ a protocol in which successive infusions are delivered in a 1–2 s pulse consisting of 20 μ l of the test solution. Alternatively, continuous infusion of a test solution can be automatically controlled by a syringe pump and timer (Kashinsky et al., 1990). The only limitation on chemosensory solutions that may be delivered by this infusion system is fluid viscosity; we have infused solutions of isotonic saline; sucrose; quinine hydrochloride; novel olfactorants such as lemon and citral; and biologically relevant fluids such as milk, amniotic fluid, and dimethyl disulfide to fetuses as young as 17 days of gestation. If two cannulas are installed along the midline of the tongue (a procedure we have employed in conditioning experiments), it is possible to coordinate infusion of two different test solutions.

The three alternative methods for presenting chemical stimuli to the fetus offer unique advantages. The least invasive method of exposing fetuses to chemical stimuli is through manipulation of maternal diet. This approach also provides the least control over parameters of stimulus exposure to the fetus. Intra-amniotic injection results in chemical stimulation that persists within the closed environment of the amniotic sac for variable periods ranging from minutes to days. This method is appropriate for ensuring prenatal exposure to a stimulus in experiments calling for testing at a later point in gestation (Smotherman & Robinson, 1985) or after birth (Smotherman, 1982a; Stickrod, Kimble, & Smotherman, 1982) or for manipulating the chemical context in which other aspects of fetal behavior are assessed (Smotherman & Robinson, 1988a). Intraoral infusion of test solutions is better suited for direct measurement of immediate fetal responsiveness to chemical cues. The infusion technique has been applied to studies of fetal learning, habituation, sensory acuity, and stimulus-evoked motor development (Smotherman & Robinson, 1987a, 1988a). The evident drawback with intraoral infusion is the necessity to test fetuses *Ex Utero*, prohibiting longitudinal study.

MEASUREMENT OF FETAL BEHAVIOR

With the foregoing emphasis on technical procedures for preparing the pregnant rat and the fetus for observation, altering the sensory environment of the fetus, and manipulating fetal physiology, one must not neglect to devote equal consideration to problems of definition and measurement of behaviors produced by the fetus. We employ a system of observing and recording fetal behavior that exhibits the following key features. (1) Categories of fetal movement are defined in operational terms of the movements themselves. Specifically, behavioral categories are defined by the anatomical region of the body in which a particular movement occurs (e.g., foreleg, rearleg, head, mouth, trunk). Each movement is considered as an instantaneous point event.

(2) Every behavioral performance by the subject is scored, not just a focal subset of events relevant to a specific research question. Because categories are independently defined, it is possible for two or more categories to occur simultaneously. (3) Each act, comprising one or more categories of movement, is entered into an event recorder that preserves the time of data entry (± 1 s). The product is a continuous record of fetal motor activity during an observation session that preserves temporal and sequential information. (4) Fetuses are simultaneously videotaped during observation sessions. A permanent videographic record augments the basic event record and permits retrospective analysis of fetal behavior. This observation and recording protocol is consistent in its results: We have reported inter-rater reliabilities in excess of .90 when different observers are asked to detect and categorize fetal movement events. Successive observations of videographic records by the same observer result in higher reliability scores.

Clearly, an exhaustive behavioral record, even over a limited period of observation, must be reduced to describe fetal behavior or assess experimental effects. The strategy we have employed in data reduction is to construct several standard data summaries and then employ a battery of analytical techniques to address specific behavioral questions. The standard summaries provide information about the overall activity of the fetus, the frequency of events in specific behavioral categories, and the incidence of two or more independent categories of movement occurring synchronously as a single event (Smotherman, Richards, & Robinson, 1984). Summaries of overall activity, category frequency, and incidence of synchronous movements can be integrated to provide additional information about behavioral organization and repertoire diversity (Smotherman & Robinson, 1986a; Robinson & Smotherman, 1987, 1988). The frequency of activity, or of specific categories of movement, is further reduced by parsing events among successive intervals of time during an observation session; depending upon the interval selected (ranging from 5 to 60 s), relatively fine or coarse descriptions of temporal changes in behavior are obtained. Temporal summaries provide the basis for a variety of analytic procedures, including tests for bout structure (aperiodic temporal clustering of events), cyclic motor organization (Smotherman, Robinson, & Robertson, 1988), and sequential organization (Robinson & Smotherman, 1988). Temporal summaries also enable measurement of abrupt changes in fetal behavior coincident with acute changes in the fetal environment, such as produced by stimulus infusion or umbilical cord occlusion (Smotherman & Robinson, 1987b, 1988a). See Robinson and Smotherman (1988), for more detailed descriptions of these analytical procedures.

CONCLUDING REMARKS

Improved techniques for preparing the mother and fetus, manipulating the intra-uterine environment, and measuring behavior of the fetus are opening a window on a portion of the lifespan that has heretofore received comparatively little attention (Smotherman & Robinson, 1988c). Studies founded on this technology are continuing to document how some elements of the fetal repertoire promote adaptation of the fetus to changes in the intrauterine environment (Smotherman & Robinson, 1986a;

1987b) while other elements are coextensive with postnatal behavior (Smotherman & Robinson, 1987a; 1988d). As mentioned above, rat fetuses exhibit a remarkably consistent behavioral response to brief occlusion of the umbilical cord. The behavioral hyperactivity and cardiac deceleration that characterize the fetal response to hypoxia are exactly opposite to the response of newborn rat pups that are deprived of oxygen (Eden & Hanson, 1987; Smotherman & Robinson, 1988e). Because the fetal hypoxic response is evident only during the prenatal period upon manipulation of an anatomical feature unique to the fetus (the umbilical cord), we have interpreted this response as an ontogenetic adaptation.

Conversely, on day 20 of gestation rat fetuses respond to intraoral infusion of lemon with facial wiping, a stereotypic behavioral pattern that constitutes an important element of the aversive behavior of adult rats, but which, paradoxically, is not expressed during the early postnatal period. The apparent discontinuity in the development of facial wiping behavior has led us to investigate the reason for the absence of wiping during the neonatal period. Briefly, a series of experiments have now revealed that (1) fetuses near term perform facial wiping in response to lemon infusion, (2) newborn pups tested in an age-typical (terrestrial) environment do not perform wiping, (3) newborns immersed in a buoyant fluid medium do respond to infusions with facial wiping, and (4) pups immersed in a fluid but maintained in ventral contact with a submerged hard substrate do not exhibit wiping (Smotherman & Robinson, 1989). We have interpreted these findings as evidence that the early postnatal absence of facial wiping is not due to immaturity of neural substrates that subserve the wiping response or to a lack of continuity between fetal and adult aversion behavior but to the environmental constraint of the expression of facial wiping. These experiments, which not only describe the development of this form of behavior but also have implications for the conduct of developmental research in general, were predicated upon the advent of methods and research questions applied to the study of fetal behavior.

The fetus is an active organism that exists in a complex and changing environment. The fetus undergoes development in utero, but simultaneously interacts with its environment through its behavior and physiology. In this way the fetus may be viewed as an active participant in its own development and not merely a passive object in a static environment (Smotherman & Robinson, 1987c). We feel that this change in our view of the fetus is healthy. Renewed interest in fetal development is a logical extension of the epigenetic perspective of the prenatal period. Such a perspective, which constitutes the theoretical underpinnings of all developmental research, holds that ontogeny is a cumulative process. Thus, behavioral sophistication in the neonate implies that the organism begins the postnatal period with a rich behavioral and experiential history. It is our belief that more detailed knowledge of this prenatal history will foster a more complete understanding of behavioral development in general.

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