

Habituation and Classical Conditioning in the Rat Fetus: Opioid Involvements

Scott R. Robinson
William P. Smotherman
Binghamton University

There is abundant evidence that fetal rats can modify their behavior and responsiveness to sensory stimuli as a function of experience. Repeated infusion of a lemon odor solution into the fetus's mouth results in diminished behavioral and cardiac responsiveness (habituation). Pairing a chemosensory infusion with LiCl injection suppresses fetal activity upon representation of the taste/odor cue (conditioned aversion). Pairing a chemosensory stimulus with another treatment that elevates motor activity results in increased activity upon representation of the stimulus (conditioned activation). These findings have confirmed that altricial fetuses exhibit basic forms of learning during the prenatal period. Most recently, experiments have suggested that the endogenous opioid system of the fetus and neonate may play a role in facilitating or interfering with early learning. Intraoral infusion of a small volume of milk, for example, results in elevated activity at the kappa subclass of opioid receptors. Opioid activity produced by pharmacological treatment or sensory manipulation has the effect of reducing fetal responsiveness to cutaneous stimulation of the perioral region; perioral cutaneous responsiveness thus can be used as a behavioral bioassay of opioid activity in the fetus. If milk infusion is paired with a chemosensory stimulus (sucrose) or an oral tactile stimulus (artificial nipple), the fetus will exhibit reduced responsiveness in this bioassay upon reexposure to the sucrose or nipple alone. Although opioids are demonstrably involved in mediating both the unconditioned response

(evoked by milk) and conditioned response (evoked by sucrose or nipple after pairings with milk), the conditioned response involves activity in the mu opioid system, not the kappa system, suggesting that early learning promotes interactions between different opioid systems. The results of learning studies conducted with fetuses, which lack experience with milk or other suckling stimuli, have implications for understanding the sensory determinants and underlying neurobiology of behavior in the late prenatal period and during the first suckling episode after birth.

FETAL CHEMOSENSORY EXPERIENCE

Much of mammalian behavior is regulated by chemical stimuli that activate the olfactory, gustatory, or trigeminal systems. This is particularly true in young altricial mammals, which lack functional visual or acoustic senses and depend heavily on chemosensory cues in postnatal maternal-infant interactions, recognition of parents, siblings, and other kin, and the development of ingestive behavior and early dietary preferences. Most importantly, chemosensation plays crucial roles in suckling behavior and learning that occurs in the context of suckling. Odor cues on or around the nipple are important for many neonatal mammals to find and attach to the nipple (Blass, 1990); in humans, maternal breast odors facilitate recognition of the mother by the infant (Porter, 1991). Aromatic chemicals present in food or drink, such as garlic or alcohol, can be transferred to mother's milk and thereby transmitted to the breast-fed human infant, which can alter infant behavior at the nipple (Mennella & Beauchamp, 1991). Odors or tastes associated with suckling, or that are present in milk, provide the basis for learning in the neonate, which has been shown to influence adult food preferences, mate choice, and sexual behavior in rats (Galef & Henderson, 1972; Fillion & Blass, 1986).

The observation that the newborn can detect taste and odor stimuli and learn contingencies associated with suckling implies that chemosensation and the capacity to learn develop before birth. The past decade has seen rapid progress in the empirical study of fetal sensory and learning abilities. For obvious technical and ethical reasons, experimental investigation of behavioral responses to chemosensory stimuli and prenatal learning has been conducted primarily with animal subjects. Techniques that permit direct manipulation and visual observation of fetal rats and other rodents have provided a window for viewing the prenatal origins of behavior (Smotherman & Robinson, 1991a). Experiments have documented the temporal and spatial organization of nonevoked motor activity (Robinson & Smotherman, 1988), the prenatal expression of organized behavioral patterns in response to chemosensory stimulation (Robinson &

Smotherman, 1992a), and the capacity to modify responses as a function of prenatal sensory experience (Robinson & Smotherman, 1991; Smotherman & Robinson, 1987). The recognition of behavioral competence in the newborn thus is expanding its frame of reference to incorporate new findings about behavior, sensation, and learning in the fetus.

Study of learning in the fetus traces trajectories of behavioral development to their logical origins—in the prenatal period (Smotherman & Robinson, 1990). The fetus is exposed to various chemical agents that are transported across the placenta to diffuse into fetal circulation or amniotic fluid and thereby gain access to fetal chemosensory receptors (Beaconsfield, Birdwood, & Beaconsfield, 1980; Maruniak, Silver, & Moulton, 1983). But the fetus is unlikely ever to experience discrete, temporally delimited chemical stimulation in utero, ensuring that responses can be assessed to the first exposure of the fetus to various sensory manipulations. Measurement of the behavioral outcomes of sensory manipulations effectively integrates across many neural functions, including detection and processing of sensory information, acquisition, retention, and retrieval of stored information, generation of central commands for stimulus-specific behavioral responses, and control of organized motor behavior. Experimental investigation of fetal learning thus provides a method for assessing the integrated output of the central nervous system during prenatal development. Finally, practical information concerning the behavioral capacities of human infants born prematurely may be obtained from experimental study of the fetus. Fetal learning thus is not a curiosity, but an active area of research in the fields of developmental psychobiology, neurobiology, and child development.

LEARNING BY THE FETUS

Learning encompasses any long-lasting change in behavior that results from prior exposure to a sensory stimulus or configuration of stimuli. Various forms of learning have been characterized that appear to reflect increasing levels of stimulus complexity or central nervous system processing. Habituation may represent one of the simplest forms of learning, which is shared by virtually all animals (Thompson & Spencer, 1966). Habituation involves a decrement in behavioral or physiological response that occurs over a series of presentations of the same stimulus and is not due to receptor adaptation or motor fatigue. Several studies have documented waning responsiveness to repeated sensory stimulation in human fetuses. For example, heart rate decelerations (bradycardia) to acoustic or vibrotactile stimuli diminish over a series of trials (Leader, Baillie, Martin, & Vermeulen, 1982). However, less is known about whether such response

decrements are central or peripheral effects. One approach for distinguishing habituation from other forms of response decrement is to present a novel stimulus after responsiveness to an original stimulus has waned: Vigorous responding to the second stimulus can rule out motor fatigue (if the responses are similar) and sensory adaptation (if the stimuli are similar) as possible influences. A more powerful approach is provided by dishabituation: Presentation of a novel stimulus after response waning can reinstate responsiveness to the original stimulus (Groves & Thompson, 1970). A few studies of human fetuses have reported fetal responding to a second stimulus and dishabituation (Kisilevsky & Muir, 1991; Madison et al., 1986), suggesting that human fetuses may exhibit habituation during the third trimester of gestation.

Habituation to chemosensory stimulation is beyond the scope of human studies, but has been demonstrated in experiments conducted with fetal rats (Smotherman & Robinson, 1992a). Data from rat fetuses have indicated that heart rate and motor activity can vary independently, suggesting the absence of cardiosomatic coupling before birth. Changes in heart rate and motor behavior thus can provide separate measures of responsiveness in fetal subjects (Smotherman, Robinson, Hepper, Ronca, & Alberts, 1991). On the last few days of the 21.5-day gestation, rat fetuses exhibit a pronounced increase in motor activity and bradycardia when a lemon odor solution is infused into the fetus's mouth. Over a series of nine lemon infusions, both motor and cardiac responses diminish nearly to baseline levels. Presentation of a second taste/odor solution—mint—after the last lemon trial is effective in reinstating responsiveness to lemon. Dishabituation is evident at an interval (2 min) that is insufficient to promote spontaneous recovery of fetal responsiveness. The decrement in fetal response to lemon infusions therefore is a centrally mediated effect, indicative of true habituation.

Prenatal exposure to chemosensory stimuli also can produce lasting changes in fetal responsiveness. Sensitization effects may result from one or many presentations of a stimulus in utero, without the need of reinforcing stimuli. Several experiments have demonstrated that exposure to a chemosensory fluid that is injected into the amniotic fluid that surrounds the fetus is sufficient to establish a preference for the same chemosensory stimulus after birth. For instance, adult rats that had received a single intra-amniotic injection of apple juice on day 20 of gestation (E20) preferred apple juice over a control solution (maple) in a two-bottle choice test (Smotherman, 1982a). Learning through prenatal exposure would appear to be a robust effect, for exposure to aversive substances, such as alcohol (6% ethanol solution), on E21 is sufficient to eliminate aversion to alcohol odor 8 days after birth (Chotro & Molina, 1990). The ability to instill preferences or reduce aversions to chemosensory stimuli through prenatal

exposure stands in contrast to many studies of learning in young postnatal animals. In studies of learning after birth, subjects in control groups that receive presentations of individual stimulus elements (e.g., the conditioned stimulus without contingent stimulation) typically do not exhibit altered sensory responsiveness. It is unclear at present whether prenatal exposure learning is the result of mere presentation of a novel stimulus before birth, or whether the preference is established through adventitious pairing of the stimulus with an unrecognized reinforcer in utero.

Although reinforcing stimuli are not necessary for prenatal learning to occur, a number of studies have documented that fetuses have the capacity to associate independent stimulus events. Most experimental demonstrations of associative learning in the fetus have employed a conditioned aversion paradigm. Fetuses are exposed to a novel odor stimulus (the conditioned stimulus or CS) that is followed immediately by ip injection of lithium chloride. In adult rats, a single pairing of LiCl with a tastant or odorant results in reduced intake or avoidance of the CS (García, Hankins, & Rusiniak, 1974). Fetuses prepared on E20 retain information from the contingent presentation of apple juice and LiCl well into the postnatal period; rat pups conditioned in utero avoid nipples of lactating females that are painted with apple juice (Stickrod, Kimble, & Smotherman, 1982a), require more time to traverse a runway suffused with apple odor to gain access to their mother (Smotherman, 1982b), and spend less time over shavings scented with apple juice (Stickrod, Kimble, & Smotherman 1982b). Fetal rats that receive an odor-LiCl pairing on E17 exhibit changes in motor activity upon reexposure to the odor CS as fetuses on E19 (Smotherman & Robinson, 1985). Experiments such as these, which represent a form of classical conditioning, have firmly established that the fetus is capable of associative learning during the prenatal period.

FETAL LEARNING IN AN ECOLOGICAL CONTEXT

Although effective, the first demonstrations of fetal learning employed paradigms that involved somewhat arbitrary and artificial stimuli that were not clearly related to contingencies that the fetus or neonate might experience in its life history. These experimental approaches more recently have given way to paradigms employing stimuli of ecological relevance to the fetus or neonate. Hepper (1988), for example, has reported that introducing novel food items into the diet of pregnant rats can expose the fetus to specific chemosensory compounds (such as the aromatic sulfur compounds in garlic) that are transported across the placenta and gain access to fetal chemosensory receptors. Rat pups that are exposed in utero to garlic via maternal diet express a preference for the odor of garlic after

birth. Cross-fostering to mothers that had no exposure to the garlic diet had no effect on the amount of time spent by pups over the garlic odor, suggesting that prenatal experience with garlic compounds was responsible for the garlic preferences expressed by pups.

Although on the surface it appears that prenatal exposure learning can occur in the absence of reinforcement, fetuses may experience conditions in utero that function like reinforcing stimuli. Hypoxia, which can occur under conditions of normal intrauterine development, is an example of a prenatal event that can support classical conditioning in the fetus. Under natural circumstances, hypoxia can occur when the umbilical cord becomes twisted or is otherwise occluded (Mann, 1986). Transient hypoxic episodes, induced by clamping the umbilical cord, evoke stereotypic behavioral responses in rodent fetuses (Robinson & Smotherman, 1992b), and can alter subsequent responsiveness to chemosensory stimuli present at the time of umbilical cord compression (Robinson & Smotherman, 1991). Changes in responsiveness to an odor present in the amniotic fluid can be conditioned by pairing the odor cue with the onset of hypoxia (resulting in an odor aversion, Hepper, 1991, or cessation of cord compression (resulting in an odor preference, Hepper, 1993). These experimental findings suggest that events that occur just before or at the time of birth can function as reinforcers to support classical conditioning.

Learning that occurs during the few hours before and after birth appears to play an important role in the natural history of the rat. During parturition, the mother engages in self-grooming and licking that have the effect of depositing amniotic fluid on her ventrum and nipples. The chemosensory properties of amniotic fluid, or of specific constituents of the fluid, serve to direct the first nipple attachment of the newborn rat (Pedersen & Blass, 1982). Subsequent to the initial attachment, other chemosensory cues regulate nipple attachment, including pup saliva that is deposited on nipples during suckling. The influence of amniotic fluid and pup saliva on nipple attachment may be attributed to exposure learning or conditioning that occurs around the time of birth.

The simple sulfur compound dimethyl disulfide (DMDS) is one component of pup saliva that is effective in directing nipple attachment in pups with suckling experience (Pedersen & Blass, 1981). Because DMDS is produced in the salivary glands of the newborn, it may be present in utero. Other sulfur compounds have been identified in the composition of milk and can be introduced into amniotic fluid via maternal circulation and transport across the placenta. Moreover, carbon disulfide (CS₂) is a constituent of the breath of rats that facilitates social transmission of dietary information in juvenile and adult rats (Galef, Mason, Preti, & Bean, 1988). An intriguing possibility suggested by these experimental findings is that simple chemical odorants that are present in body fluids or tissues, such as

these sulfur-based compounds, may promote learning about food-related stimuli in the adult feeding or neonatal suckling situations. The reinforcing properties of such compounds may be related to their ability to engage different neurochemical systems of the fetus or pup. Brief exposure of the E21 rat fetus to DMDS, for instance, results in enhanced activity in the endogenous opioid system (Smotherman & Robinson, 1992b).

FETAL OPIOID SYSTEM: EFFECTS ON BEHAVIOR AND SENSORY RESPONSIVENESS

Since the discovery of endorphins in the 1970s, the endogenous opioid system has been found to encompass a number of classes of receptors and their associated ligands (Kosterlitz, 1991). Multiple classes of receptors have been identified and related to different biochemical, physiological and behavioral functions in the rat. In vitro studies have indicated that receptors for two opioid systems—the mu and kappa systems—are present in the brain and spinal cord during the prenatal period. The presence of precursor molecules, opioid peptides, and receptors of both the kappa and mu systems by midgestation (Leslie & Loughlin, 1993) suggests that these opioid systems are functional and may influence behavior in the fetus.

Initial investigations of the effects of opioids on fetal behavior administered opioid drugs, such as morphine, to pregnant rats and recorded changes in motor activity in fetal subjects (Kirby, 1981). More recent studies have administered selective opioid agonists and antagonists directly to individual fetal subjects and assessed effects on sensory responsiveness. Because conventional behavioral indices of opioid activity are impractical to employ with fetal subjects, several alternative behavioral bioassays have been developed based on neuroethological analysis of simple action patterns in the rat fetus (Smotherman & Robinson, 1992c, 1992d). One behavioral bioassay that has proven particularly sensitive to opioid manipulations is the facial wiping response evoked by perioral cutaneous stimulation (Smotherman & Robinson, 1992c). The stimulus in this bioassay is presented by applying a stiff bristle (von Frey filament) near the corner of the fetus's mouth. On E20 and E21, 70–80% of untreated fetal subjects respond to the perioral probe by performing one or more wiping strokes in which the forepaw is moved from ear to nose in contact with the face. Administration of mu or kappa opioid agonists, such as morphine, DAMGO (selective for mu receptors), or U50,488 (selective for kappa receptors) results in dose-dependent decreases in facial wiping expressed in this bioassay. The effects of opioid agonists can be blocked if subjects are treated with receptor-specific antagonists, such as β -funtrexamine HCl (FNA) or [Cys², Tyr³, Orn⁵, Pen⁷]amide (CTOP) for mu

receptors, or nor-binaltorphimine diHCl (BNI) for kappa receptors, prior to agonist administration (Smotherman, Moody, Spear, & Robinson, 1993; Smotherman, Simonik, Andersen, & Robinson 1993). The effect of agonist and antagonist drugs on fetal responsiveness, as measured in the bioassay of perioral cutaneous sensitivity, indicates that the mu and kappa opioid systems are sufficiently mature to mediate changes in behavior in the late prenatal period.

The ability to produce behavioral effects with opioid drugs is evidence for one dimension of functionality around the time of birth, but implies little about the role of the endogenous opioid system in the regulation of fetal or neonatal behavior. Certain stimulus manipulations that mimic features of the postnatal environment are effective in evoking activity in the fetal opioid system. For instance, a single 20- μ l infusion of milk into the mouth of the E21 fetus nearly eliminates the facial wiping response as expressed in the bioassay. The milk-induced reduction in fetal responsiveness is opioid-mediated and can be blocked by pretreatment with naloxone or the kappa-selective antagonist BNI (Smotherman & Robinson, 1992c). Reduced responsiveness is most pronounced when the perioral probe is applied 1 min after infusion, is still evident at 3 min, and dissipates approximately 5 min after exposure to milk. The finding that milk promotes activity in the kappa opioid system has been replicated in subsequent experiments with the bioassay of perioral sensitivity (Robinson, Moody, Spear, & Smotherman, 1993) and implicated in other metrics of fetal behavior (Smotherman & Robinson, 1992d). Exposure to milk has been reported to promote opioid activity in newborn rats delivered by caesarean section and tested before contact with the mother (Blass, Jackson, & Smotherman, 1991), and milk sucrose, corn oil, or other sapid solutions promote opioid-like behavioral effects in older rat pups (Blass & Fitzgerald, 1988; Shide & Blass, 1989, 1991); activation of the endogenous opioid system has been inferred from experiments with human infants (Blass, 1990). However, mono- or disaccharide solutions, lipids, or other chemosensory stimuli do not appear to affect opioid activity in the fetus. Apart from milk, only one other chemical stimulus—DMDS—has been found to evoke an opioid response, and like milk, this simple sulfur compound promotes activity in the kappa system (Smotherman & Robinson, 1992b). The ability of milk or DMDS to engage the endogenous opioid system of the fetus is evident upon the fetus's first exposure to these stimuli, indicating that suckling or specific chemosensory experience is not necessary for the expression of sensory-evoked opioid activity. Moreover, milk continues to evoke opioid activity after a series of infusions, suggesting that this form of fetal responsiveness does not quickly habituate.

Less information is available about the influence of endogenous opioids on fetal behavior in the absence of eliciting stimuli. Administration of

naloxone to unmanipulated rat fetuses has modest effects on overall motor activity on E21, but not E20 (Smotherman & Robinson, 1992c). Similarly, BNI but not FNA or CTOP produces a slight increase in motor activity on E21, suggesting that the kappa opioid system exhibits spontaneous activity near term in the fetal rat. Studies of chemosensory habituation to repeated lemon infusions also have implied endogenous opioid activity in the E21 fetus. The expression of facial wiping quickly wanes in control subjects over a series of lemon infusions, and pretreatment with naloxone or BNI has little effect on the initial responsiveness of fetuses or the rapidity of response waning. Opioid blockade also does not affect the responsiveness of fetuses to a novel chemosensory or tactile stimulus presented 1 min after the infusion series. Pretreating fetal subjects with naloxone or BNI results in a significant increase in facial wiping upon representation of the original lemon stimulus. Moreover, novel chemosensory and tactile stimuli are both effective in reinstating responsiveness to lemon, suggesting that blockade of endogenous opioid activity facilitates dishabituation. Pretreatment with FNA or CTOP, however, does not appear to influence initial responsiveness, habituation, or dishabituation. Habituation is thought to represent a central process that regulates attention to novel and familiar stimulation (Groves & Thompson, 1970; Thompson & Spencer, 1966). Because fetuses are less attentive to changes in sensory stimulation as a result of endogenous opioid activity, the kappa opioid system may play a role in regulating selective attention and stimulus processing in the near-term fetus.

CLASSICAL CONDITIONING IN THE FETUS

In many respects, information about the learning abilities of the fetus has been guided by technologies and experimental procedures available to investigators. Nearly all earlier studies of associative learning, for example, employed experimental designs that involved exposing the fetus to one or more training trials at one gestational age and evaluating the effects of conditioning later in gestation or after birth (Smotherman & Robinson, 1987). This strategy, although sufficient to document that learning can occur, introduces confounds that limit the types of conclusions that may be drawn from data obtained in developing animals. One assumption that is implicit in studies of adult learning is that the behavioral repertoire of the subject changes little between the time of training and testing. Changes in behavior that are expressed upon reexposure to the CS therefore may be concluded to be the result of learning. Such an assumption is unwarranted in studies of developmental learning, because training and testing are superimposed on sensory, motor, and central neural systems that are

rapidly differentiating, with the consequence that the behavioral repertoire can change dramatically over the span of a few days. Many of the experimental confounds that are intrinsic to developmental study may be circumvented by employing a conditioning paradigm in which training and testing are implemented at one age within a single experimental session.

The conditioned activation of fetal behavior provides an example of how associative learning may be investigated in a single-session paradigm (Smotherman & Robinson, 1991b). Experimental subjects receive a series of four paired infusions of a CS (sucrose) followed immediately by infusion of lemon (the unconditional stimulus, US), which evokes an increase in overall motor activity. This classical conditioning procedure results in an increase in fetal activity upon representation of sucrose 6 min after the last training trial. Various control groups represented within the same pregnancy confirm that the change in fetal response to sucrose is not a result of sensitization effects due to repeated exposures to the CS alone, residual effects of repeated presentation of the US, or nonassociative effects as indicated by explicitly unpaired presentations of the CS and US. Because training and testing are conducted at a single gestational age, it is unlikely that changes in the behavioral repertoire contribute to the behavioral activation expressed by fetuses exposed to CS-US pairings. Changes in fetal motor activity therefore reflect associative learning of the contingency between CS and US presentations during training. Because it avoids confounds that arise from training and testing subjects at different ages, the single-session paradigm for classical conditioning represents an advance in the ability to study age-related changes in learning capacities during the prenatal period. It also offers the possibility of investigating neural mechanisms that subserve sensory and learning processes in the fetus through administration of neuroactive substances at the time of training or testing.

CONDITIONED OPIOID ACTIVITY: A MODEL OF THE FIRST SUCKLING EPISODE

A number of studies have implicated different neurotransmitter or neuromodulator systems in the expression of learning early in development. Of particular interest is the endogenous opioid system, which appears to play a role in the reinforcing effects of milk. Milk has been shown to support both classical conditioning (Johanson & Teicher, 1980; Brake, 1981) and appetitive learning (Johanson & Hall, 1979) in neonatal rats in experiments conducted only a few days after birth. Exogenous administration of morphine during exposure to a novel odor can produce conditioning effects similar to milk, with low doses promoting a preference for the

odor (Kehoe, 1988). Similar experiments with selective agonists suggest that pharmacological activation of kappa opioid receptors results in conditioned odor preferences in 3-day-old rat pups, but conditioned aversions for the odor in 7-day-old and adult rats (Barr, 1993). Although the critical experiment, in which naloxone is administered to block the reinforcing effects of milk, apparently has not been conducted in rat neonates, circumstantial evidence suggests that milk engages the endogenous opioid system, which functions to reinforce learning in the neonate (Shide & Blass, 1991). Opioid activity also can be expressed as part of the conditioned response. After a novel odor is paired with an injection of morphine, reexposure to the odor results in diminished sensory responsiveness (increased latency to withdraw a paw from a heated surface), which is reversible with naloxone. Associative learning supported by pharmacological activation of the opioid system during training thus can promote conditioned opioid activity at the time of testing (Kehoe & Blass, 1989).

The functional relationship between milk, opioid activity, and learning recently has been explored in more detail in a series of experiments conducted with E20 rat fetuses (Arnold, Robinson, Spear, & Smotherman, 1993; Robinson, Arnold, Spear, & Smotherman 1993). These experiments have employed a single-session conditioning paradigm to determine whether pairings of milk with tactile or chemosensory cues can support classical conditioning in the fetus. Following a research strategy that has proven successful in studies of learning in neonatal subjects (e.g., Johanson & Terry, 1988), stimuli and response measures were selected to be developmentally appropriate for the behavioral capacities of fetuses. Specifically, the CS in these experiments was an artificial nipple, fashioned from soft vinyl in the approximate dimensions of a nipple from a lactating rat, which was presented to the fetus by gently touching the nipple to the external aperture of the mouth. Rat fetuses are responsive to the artificial nipple, exhibiting a variety of specific motor patterns including mouthing, licking, forelimb treadling and oral grasping of the nipple (Robinson et al., 1992). Fetal subjects are conditioned in a series of three training trials, in which the nipple (CS) is presented for a brief period and immediately followed by milk infusion (US). Fetuses exposed to nipple-milk pairings during training and reexposed to the nipple alone during testing exhibit reduced responsiveness in the bioassay of perioral cutaneous sensitivity (Fig. 16.1). No reduction in perioral responsiveness is evident among fetuses in control groups that are exposed during training trials to the nipple alone (CS sensitization controls), milk alone (US sensitization controls), or the artificial nipple several minutes after each milk infusion (unpaired exposure controls). The same pattern of results has been obtained from experiments employing a chemosensory CS (sucrose) paired with milk. These experimental findings indicate that presentation of the CS

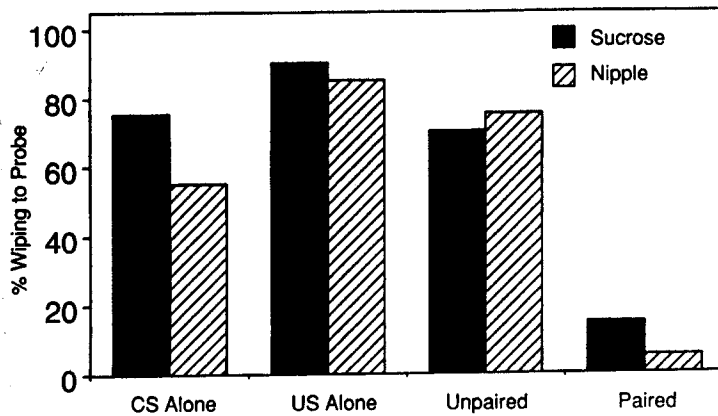


FIG. 16.1. Experimental stimuli in fetal conditioning experiments consisted of presentation of an artificial nipple or intraoral infusion of sucrose as the conditioned stimulus (CS) and intraoral infusion of milk as the unconditioned stimulus (US). Different groups of fetal subjects were exposed to the CS alone, US alone, unpaired presentations of the US and CS, or paired presentations of the CS and US in a series of three conditioning trials separated by 5-min intervals. At min 18, all subjects were reexposed to the CS, and 1 min later were exposed to a perioral tactile stimulus to assess cutaneous sensitivity. This figure depicts the percentage of fetal subjects that exhibited a facial wiping response in the bioassay after reexposure to the sucrose or artificial nipple (CS).

during testing does not in itself alter fetal responsiveness in the bioassay, and confirms that contingent presentations of the artificial nipple (or sucrose) and milk can support classical conditioning of sensory responsiveness in the rat fetus.

A single milk infusion promotes opioid activity that reduces fetal responsiveness in the bioassay, and reexposure to the artificial nipple after nipple-milk pairings elicits a conditioned response that is expressed as altered cutaneous sensitivity. Reexposure to the nipple therefore may evoke a conditioned increase in activity within the endogenous opioid system. The hypothesis of conditioned opioid activity has been tested by administering naloxone after the series of training trials and before reexposure to the CS. Fetuses that experienced unpaired presentations of milk and the nipple exhibit high levels of facial wiping in the bioassay after reexposure to the nipple, regardless of naloxone treatment. Fetuses that receive a control injection of saline after nipple-milk pairings exhibit reduced responsiveness during testing, but the responsiveness of naloxone-treated subjects remains high, indicating that blockade of opioid activity before testing eliminates the expression of conditioned changes in fetal cutaneous sensitivity (Fig. 16.2). Administration of selective opioid antagonists before testing has confirmed that contingent presentations of the

nipple and milk support the classical conditioning of opioid activity in the fetus. However, the CS-evoked reduction in fetal responsiveness is blocked with FNA or CTOP, not BNI, suggesting that the conditioned opioid response involves activity at mu, not kappa, opioid receptors (Fig. 16.2). The mu opioid system, which does not play a role in the initial responsiveness of fetuses after milk infusion, comes to be accessed by the artificial nipple after its association with milk.

Attachment to the nipple is one of the first critical actions that the newborn rat must perform. The opportunity to attach to the nipple and engage in sucking behavior, whether nutritive or nonnutritive, can serve as an effective reinforcer in both classical and instrumental learning paradigms applied to infant mammals (Amsel, Burdette, & Letz, 1976; Kenny & Blass, 1977). Furthermore, learning that takes place in the context of suckling appears to influence the development of nipple preferences in neonates, dietary preferences and kin recognition in juveniles, and reproductive behavior in adult rats. The ability of milk to engage the endogenous opioid system has been suggested as an important aspect of the reinforcing properties of milk (Shide & Blass, 1991). The experimental demonstration that pairing an artificial nipple with milk results in condi-

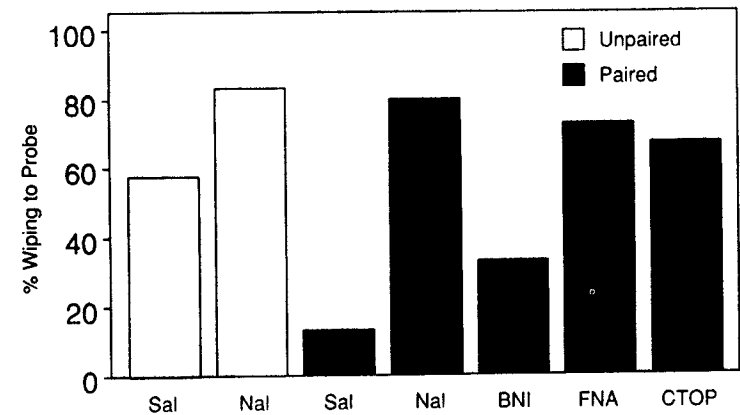


FIG. 16.2. Fetuses were exposed to the artificial nipple (CS) and milk infusion (US) in a series of three unpaired or paired presentations during training. Subjects were reexposed to the CS 10 min later. To assess opioid activity after reexposure to the CS, subjects in unpaired or paired groups received an ip injection of saline (Sal), naloxone (Nal), β -funaltrexamine (FNA) or CTOP (both mu antagonists), or nor-binaltorphimine (BNI, a kappa antagonist) 5 min before representation of the CS. The figure depicts the percentage of fetal subjects that exhibited a facial wiping response in the bioassay, which was administered 1 min after CS reexposure. Naloxone and the two mu antagonists were effective in reversing the effects of conditioning and reinstating facial wiping responses to the perioral cutaneous stimulus.

tioned opioid activity in the rat fetus, which lacks experience with milk, the nipple, or other suckling stimuli, provides further support for the pivotal role of opioids in early learning. Specifically, opioid conditioning in the fetus suggests that stimuli associated with milk during suckling can quickly come to engage opioid activity in advance of milk letdown in neonatal rats after only a few experiences at the nipple. Conditioned opioid activity evoked by the nipple or other suckling stimuli has been postulated as an important regulator of the sequence and timing of behavioral responses expressed by newborns during a suckling episode (Smotherman & Robinson, 1992e).

OPIOID INVOLVEMENT IN LEARNING AT THE NIPPLE

Through much of the recent history of research on the endogenous opioid system, opioids have been associated with subjective constructs such as the regulation of central affect or the perception of pleasant or painful qualities of a stimulus. It is clear from studies of fetal and neonatal animals that opioids also influence various forms of learning, including habituation, classical conditioning and appetitive learning (Kehoe, 1988; Smotherman & Robinson, 1993). Both the mu and kappa opioid systems attain a peak in receptor density in the perinatal period. Moreover, the endogenous opioid system can be engaged by a number of stimuli that are present in the environment of the newborn, such as milk, DMDS, and placental tissue (Kristal, 1991), and through association with these stimuli may be activated by other features of the suckling situation, such as the tactile or olfactory characteristics of the nipple. The numerous examples of interaction between ecologically relevant stimuli and endogenous opioid activity imply that opioids have functional significance in the early development of behavior.

One of the commonalities in the behavioral effects of opioids in the fetus is the change in behavioral organization and responsiveness associated with opioid activity. Organizational changes promoted by milk or opioid treatment resemble transitions between conventional behavioral states (Smotherman & Robinson, 1992e; Robinson & Smotherman, 1992c), which are associated with differences in the processing of sensory information and motor output. The learning process also has the effect of reducing the degrees of freedom in both sensory and behavioral domains: A few stimuli come to evoke specific behavioral responses. Particularly in young animals, behavioral development often proceeds by stabilizing many behavioral variables, which facilitates organized change in the few remaining

variables (Golani & Fentress, 1985). It may be parsimonious to conceive of the reinforcing properties of opioid activity as a neurochemical mechanism for altering the way infants interact with their sensory environment. For instance, kappa opioid activity effected by pharmacological treatment does not uniformly suppress fetal motor activity and sensory responsiveness. Rather, kappa activity results in increased organization in motor activity, reduced responsiveness to perioral cutaneous stimuli, and increased responsiveness to intraoral chemosensory cues (Smotherman & Robinson, 1992d). Similarly, peripheral administration of mu-agonist drugs has little effect on fetal motor behavior and simultaneously reduces responsiveness to some cutaneous stimuli (e.g., perioral tactile probe; Smotherman, Simonik, Andersen, & Robinson, 1993) while increasing responsiveness to others (e.g., artificial nipple; Robinson et al., 1992). Opioid activity thus may facilitate learning by introducing sensory and behavioral stability in young animals that are surrounded by novel stimuli—the proverbial “blooming, buzzing confusion” referred to by William James (1890/1952). By redirecting and focusing responsiveness to a subset of available stimuli, activation of the endogenous opioid system may serve as an ontogenetic adaptation that promotes selective attention and facilitates learning early in development.

FUTURE DIRECTIONS

The empirical findings presented in this chapter are part of an ongoing program of research into the prenatal origins of motor behavior, sensory responsiveness, and learning. Experiments examining conditioned changes in the endogenous opioid system of the fetus have united several different lines of research in our laboratory and will serve as foci for further investigation. Learning experiments with fetal rats have corroborated that chemical senses develop and begin to exhibit function earlier than other sensory modalities (such as vision and audition), raising the question of whether presentation of stimuli in a chemosensory modality offers advantages for the investigation of prenatal sensory and learning capacities. The study of learning in neonatal animals has undergone a steady transformation from the use of artificial stimuli (such as electric shock and injection of illness-inducing chemicals) to contingencies that are relevant to the ecology of the developing mammal (such as odor cues and intraoral infusion of milk). Future investigation of learning in the fetus may also profit from the selection of unconditioned and conditioned stimuli that mimic environmental features that are important to the fetus or to the newborn. Much of the learning in the neonate appears to occur in settings, such as suckling,

that affect the endogenous opioid system. Further studies will explore the role of opioid activity in regulating responsiveness to sensory stimuli and promoting learning in developing animals. Neurobiological substrates of learning typically are studied in animals that have relatively simple nervous systems, such as invertebrates. Investigation of learning in rodent fetuses may provide an analogous simple system for understanding the neural basis for learning and memory, but one that has more direct applicability for behavior in adult mammals. Ultimately, the broad aims of this basic research are to provide insights or suggest hypotheses that are relevant for the human fetus, premature infant, and full-term newborn. We expect that experimental study of fetal learning and behavioral plasticity will serve as a proving ground for potential therapies aimed at improving the health and development of premature infants and other infants at risk.

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Edited by

Jean-Pierre Lecanuet
*Centre National de la Recherche Scientifique
Ecole Pratique des Hautes Etudes*

William P. Fifer
Columbia College of Physicians and Surgeons

Norman A. Krasnegor
*National Institute of Child Health and Human
Development, National Institutes of Health*

William P. Smotherman
Binghamton University



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