Toward a Neurobiology of Attachment

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ABSTRACT New laboratory research has begun to reveal a network of simple behavioral, physiological, and neural processes that underlie the psychological constructs of attachment theory. It has become apparent that the unique features of early infant attachment reflect certain unique features of early infant sensory and motor integration, early learning, communication, motivation, and the regulation of biobehavioral systems by the mother–infant interaction. This chapter is organized around the three major questions that gave rise to the concept of attachment: How does the infant find its own mother and stay close to her? Why does separation of the infant from its mother produce such severe physiologic and behavioral responses? How can individual differences in adult offspring and especially in their maternal behavior be related to the patterns of their early life with their parents? In each of these cases, we review the recent research that has given us new answers to these questions at the level of early behavioral and cognitive processes and their neurobiological substrates. Attachment remains useful as a concept, like hunger, that describes the output of subprocesses that work together within the frame of a vital biological function.

The word attachment has come to refer to a broad range of behavioral processes and mental states unified by a single central concept. Attachment theory envisages a unique motivational system with evolutionary survival value, on a par with hunger and sex, that is organized to maintain physical proximity to the mother/caregiver soon after birth and psychological proximity, or closeness, later in development (Bowlby, 1969). The special qualities of this bond reflect the nature of the infant’s and the caregiver’s mental representations of the behavioral dynamics and patterns of their many previous interactions. Much current research is concerned with identifying developmental continuities between individual differences in the patterns of parent–infant attachment and the later cognitive and emotional characteristics of the child and adult. In an extension of these longitudinal correlations, there is evidence that attachment experiences in infancy strongly affect characteristics of the attachment pattern of the mother with her infant in the next generation. Several extensive reviews of this field have recently been published, covering human and nonhuman primate research (Cassidy and Shaver, 1999; Goldberg, Muir, and Kerr, 1995; Suomi, 1997) and the growing field of neurobiological studies (Carter, Lederhandler, and Kirkpatrick, 1997; Insel, 1992; Kraemer, 1992; Wilson and Sullivan, 1994).

It is curious that most of the research on attachment subsequent to Bowlby’s landmark volume defining the field (Bowlby, 1969) has focused on the different patterns of early attachment and their later developmental correlates, leaving far less studied the developmental processes through which attachment is initially formed in altricial (slow-developing) mammals and the behavioral mechanisms underlying the dramatic responses of infants to separation. Meanwhile, others interested in processes underlying the early development of motor and sensory systems, perception, attention, learning, memory, communication, motivation, and emotion have developed new methods (Shair, Barr, and Hofer, 1991) and a knowledge base in each of these areas (Michel and Moore, 1995) without relation to the concept of attachment or its field of research. To these researchers, the range of associated phenomena did not appear to function as a unitary system and attachment concepts were too global to generate incisive research questions that could lead to a deeper understanding of the phenomena described.

Now that a knowledge base has been established in the various research areas just described, at least for one mammalian species, the laboratory rat, we can revisit the basic observations on which attachment theory is based, and describe some of the underlying processes responsible for them in terms that can be related to neural structure and function. When one does this, it becomes apparent that the unique features of early attachment phenomena can be shown to be the result of certain unique features of early sensory and motor patterns.
integration, of early learning, communication, motivation, and the regulation of biological and behavioral systems by the mother–infant interaction. The apparently unified nature of attachment and its function as a system are the result of the fact that for the mammalian fetus and infant, the entire environment virtually begins and ends within the confines of a unified source of stimulation, the mother. Thus attachment, like hunger, is made up of a number of subprocesses, and exists as a useful concept because it describes the output of these subprocesses as they work together within the larger scale arena of social relationships.

The observations that attachment theory was formulated to explain are used to organize this chapter. In each case, we describe recent evidence for the behavioral and physiological processes that underlie the concepts of attachment theory and relate these to the neural processes found to be responsible in laboratory studies thus far. Because we know a great deal less about the neurobiological mechanisms than about the cognitive and emotional processes, our aim is to suggest where neurobiological research methods can be used to explore this new research field.

Three major questions are raised by the basic observations that led to the field of attachment research. First, how does the infant come to know and prefer its own mother, maintain proximity with her, and continue to do so, even despite abuse and neglect at her hands? Second, why does separation from the mother produce such intense and widespread emotional responses in the infant? Third, how do individual differences arise in the characteristic patterns or qualities of the mother–infant interaction and how do these early interactions become translated into long-term effects on infant development and ultimately into similar maternal behavior in the next generation? We focus on those areas in which we have the most detailed knowledge of the underlying cognitive and neurobiological processes involved, work almost exclusively done with laboratory rats. However, we relate these to the human wherever possible. We describe in some detail the rapid and highly specific early olfactory learning processes recently discovered in infant rats and their neural substrates. This phenomenon appears to represent an altricial mammalian counterpart of avian imprinting, and some recent evidence suggesting a similar process at work in newborn babies is described.

Initial formation of a specific attachment

Infants of mammalian species that are born in an immature state, such as the human and the laboratory rat, face a daunting cognitive task. They must eventually learn to identify, remember, and prefer their own mother; and they must learn to use these new cognitive capacities to reorganize their simple motor repertoires, long adapted to the uterine environment, so as to be able to approach, remain close, and orient themselves to their mothers for the first nursing bout. It has been assumed until recently that these processes were well beyond the capacities of newborn mammals (except in precocial species such as the sheep) and that the relationship initially depended almost entirely upon maternal behavior until well into the nursing period (Bowlby, 1982; Kraemer, 1992). Attachment has thus been supposed to be built up slowly in the weeks or days after birth in human or rat. But the last decade has produced a number of studies revealing earlier and earlier evidence of learning, even extending into the prenatal period. In addition, coordinated motor acts have been demonstrated in fetuses in response to specific stimuli that will not be encountered until after birth. Thus the solutions for the infant’s cognitive tasks appear to be found much earlier than previously thought and to take place through novel developmental processes that had not been imagined until recently.

Prenatal origins The first strong evidence for fetal learning came from studies on early voice recognition in humans, in which it was found that babies recognize and prefer their own mother’s voice, even when tested within hours after birth (de Casper and Fifer, 1980). Bill Fifer continued these studies in our department using an ingenious device through which newborns can choose between two tape-recorded voices by sucking at different rates on a pacifier rigged to control an audio tape player (reviewed in Fifer and Moon, 1995). He has found that newborn infants, in the first hours after birth, prefer human voices to silence, female voices to male voices, their native language to another language, and their own mother to another mother reading the same Dr. Seuss story. In order to obtain more direct evidence for the prenatal origins of these preferences (rather than very rapid postnatal learning), Fifer filtered the high-frequency components from the tapes to make the mother’s voice resemble recordings of maternal voice by hydrophone placed within the amniotic space of pregnant women. This altered recording, in which the words were virtually unrecognizable to adults, was preferred to the standard mothers’ voice by newborns in the first hours after birth, a preference that tended to wane in the second and third postnatal days. Furthermore, there is now evidence that newborns prefer familiar rhythmic phrase sequences to which they have been repeatedly exposed prenatally (DeCasper and Spence, 1989).
In a striking interspecies similarity, rat pups were subsequently shown to discriminate and prefer their own dams' amniotic fluid in preference to that of another dam when offered a choice in a head-turning task (Hepper, 1987). Newborn pups were also shown to require amniotic fluid on a teat in order to find and attach to it for their first nursing attempt (Blass, 1990). Robinson and Smotherman (1995) have directly tested the hypothesis that pups begin to learn about their mothers' scent in utero. They have been able to demonstrate one-trial taste aversion learning and classical conditioning in late-term rat fetuses, using intraoral cannula infusions and perioral stimulation. Taste aversions learned in utero were expressed in the free feeding responses of weanling rats nearly 3 weeks later. They went on to determine that aversive responses to vibrissa stimulation were attenuated or blocked by intraoral milk infusion, a prenatal "comfort" effect they found to be mediated by a central kappa-opioid receptor system. Interestingly, when artificial nipple stimulation was presented as a predictive cue (CS) for intraoral milk in a learning paradigm, and a reduction in fetal responsiveness to the CS was acquired in this way, the conditioned response was blocked by a mu-opioid antagonist rather than a kappa antagonist. This suggests an important role for the opioid system in the organization of fetal behavior by redirecting and focusing responsiveness to a subset of available stimuli (Robinson and Smotherman, 1995).

These forms of fetal learning involving maternal voice in humans and amniotic fluid in rodents appear to play an adaptive role in preparing the infant for its first extraterine encounter with its mother. They are thus the earliest origins yet found for attachment to the mother.

The Perinatal Transition. In addition to the evidence for fetal learning just described, specific adaptive motor response capabilities have also been found in later term rat fetuses using an exteriorized in vivo preparation (Smootherman and Robinson, 1992). The reflexes of licking, mouthing, and sucking necessary for postnatal nipple grasp and nursing can be elicited in late-term fetuses, as can the characteristic stretch response to oral milk and the facial wiping (rejection) response to unfamiliar tastes that are usually associated with rat pups tested several days postnatally. The emergence of these reflex responses in anticipation of the postnatal environment in which they will be expressed provides the newborn with the components needed for the transition to postnatal life and for the rapid organization of an integrated response repertoire tuned to specific characteristics of the maternal body.

The spontaneous motor acts needed for an attachment system also appear to be developing prior to birth. Rat fetuses engage in a number of spontaneous behaviors in utero, including curls, stretches, and trunk and limb movements. These acts were observed to increase markedly in frequency with progressive removal of intrauterine space constraints, as pups were observed first through the uterine wall, then through the thin amniotic sac, and finally unrestrained in a warm saline bath (Smootherman and Robinson, 1986). When newborn pups are observed prior to their first nursing bout, they resemble exteriorized fetuses until the mother lowers her ventrum over them. Their behavior then changes rapidly over the first few nursing bouts, into the complex repertoire described below.

An Attachment System in the Newborn. When pups less than a day old are stimulated gently by soft surfaces from above, as when the mother hovers over them, they show a surprisingly vigorous repertoire of behaviors (Polan and Hofer, 1999a). These include the curling and stretching seen prenatally, but now also include locomotor movement toward the suspended surface, directed wriggling, audible vocalizations, and, most strikingly, turning upside down toward the surface above them. Evidently, these behaviors propel the pup into close contact with the ventrum, maintain it in proximity, and keep it oriented toward the surface. They thus appear to be very early attachment behaviors. In a series of experiments, we found that these are not stereotyped reflex acts, but organized responses that are graded according to the number of maternal modalities present on the surface, such as texture, warmth, and odor (Polan and Hofer 1999a). Furthermore, they are enhanced by periods of prior maternal deprivation, suggesting the rapid development of a motivational component. By 2 days of age, we found that pups discriminate their own mother's odor in preference to equally familiar nest odors (Polan and Hofer 1998) and by the first postnatal week, Hepper (1988) has shown that pups discriminate and prefer their own mother, father, and siblings to other lactating females, males, or age mates.

These results show that a highly specific "behavioral attachment system" (Bowlby, 1969), capable of approach and proximity maintenance to the mother and motivated by brief periods of separation from her, may occur much earlier in development than previously supposed. The remarkable specificity of the approach response of the infant rat to individual family members acquired within the first few postnatal days demonstrates that specificity of attachment does not require long experience or advanced cognitive and emotional
Olfaction in the rat and vision in the human provide the necessary basis for approach responses that are specific to a single individual. But this remarkable capability can develop independently of the specificity of the rat pup’s contact comfort response. For even by 2 weeks of age a rat pup will show an equal comfort response to contact with any female that is available. This nonspecificity is limited, however, and a form of “stranger anxiety” develops by the second week of life, well before weaning. Pups will avoid the odor of unfamiliar adult males (but not of familiar or unfamiliar prepubertal males), and they show immobility and a brisk adrenocortical response when the stranger is too near (Takahashi, 1992). This early fear response, like approach responses to the mother, depends upon olfactory cues (Shair et al., 1997).

Recent work in humans, inspired by these findings in lower animals, has shown that human newborns, too, are capable of slowly locomoting across the bare surface of the mother’s abdomen and locating the breast scented with amniotic fluid in preference to the untreated breast (Varendi, Porter, and Winberg, 1996). Although newborns are attracted to natural breast odors even before the first nursing bout (Makin and Porter, 1989), amniotic fluid can override this effect. Apparently, human newborns are not as helpless as previously thought, and possess approach and orienting behaviors that anticipate the recognized onset of maternal attachment at 6–8 months.

**Postnatal Learning** Although specific olfactory and/or auditory predispositions toward the infant’s own mother may be acquired prenally, after birth the newborn mammal enters a new world where contingent events, so important for more advanced forms of learning, are now occurring with great frequency. The abrupt transition from prenatal to postnatal life appears to be eased for the newborn by the presence of prenatal stimuli continuing into the postnatal environment (i.e., the odor of amniotic fluid; Lecanuet and Schaal, 1996; Mennella et al., 1995; Schaal, Marlier, and Soussignan, 1995; Varendi et al., 1998). However, it seemed likely that the neonatal capacities for stimulus discrimination and preferential approach, orienting, and proximity-maintenance behavior described in the previous section are formed by some type of rapid postnatal learning process, resembling avian imprinting, as hypothesized by Bowlby. Yet until recently, no such process had been discovered.

When developmental psychobiologists first began assessing infant rat development, any form of learning appeared beyond the capabilities of the neonatal rat. However, as our understanding of the newborn’s environment began to evolve and experimental conditions became more naturalistic, the surprising learning capabilities of rodent neonates have emerged. Since then, extensive work characterizing early learning has demonstrated that the basic laws of adult learning also apply to infant rats and that learning occurs naturally within the nest (Brunjes and Alberts, 1979; Campbell, 1984; Galef and Kaner, 1980; Leon, 1975; Miller, Jagielo, and Spear, 1989; Pedersen, Williams, and Blass, 1982; Rudy and Cheatle, 1979; Sullivan, Hofer, and Brake, 1986; Sullivan et al., 1986, 1990; Terry and Johanson, 1996).

In these studies, the use of a two-choice preference platform revealed for the first time that within 2–3 days after birth, pups are capable of learning to discriminate, prefer, approach, and maintain proximity to an odor associated with forms of stimulation that naturally occur within the early mother–infant interaction (Sullivan, Hofer, and Brake, 1986). Random presentations of the two stimuli (odor and reward) had no such effect and provided the control procedure necessary to identify the change in behavior as due to associative conditioning. Since the learning required only two or three paired presentations and since the preference was retained for many days, it seemed to qualify as an “imprinting-like process” that is likely to be central to attachment in slow-developing mammals. Indeed, a human analog of this process was found by Sullivan and colleagues (1991). These investigators presented human newborns with a novel odor, then rubbed them along their torsos to simulate maternal care; the next day, the infants became activated and turned their heads preferentially toward that odor. This suggests that rapid learning of orientation to olfactory cues is an evolutionarily conserved process in mammalian newborns.

Somatosensory information is also important in mother–infant interactions. For example, pup mortality rate (due to disturbance of nipple orientation and grasp) increases markedly when their facial somatosensory system is disrupted (Hofer, 1981). Somatosensory stimulation evokes specific orientation behaviors in 2–3-day-old pups (Polan and Hofer, 1999b), and specific contingent stimulation can be shown to alter pups responsiveness to somatosensory stimulation. Following a conditioning procedure in which whisker stimulation was paired with a reward, pups showed more vigorous responding to whisker stimulation (Landers and Sullivan, 1999a,b), resulting in enhanced responding to tactile stimulation from the mother.

From an evolutionary perspective, a reliance on learning for early attachment might appear rather risky, considering the potential for inappropriate object choice. However, considering the physical constraints
of the nest and pups' immature motor system, the range of possible attachment figures is limited. Additionally, unique characteristics of infant learning appear to greatly enhance the likelihood of pups' developing odor preferences necessary for forming maternal attachment. For example, during the neonatal period, when positive associations are readily formed, inhibitory conditioning and passive avoidance do not appear to emerge until after postnatal days 10–11 (Blozowski and Cudennec, 1980; Collier et al., 1979; Goldman and Tobach, 1967; Martin and Alberts, 1979; Stehouwer and Campbell, 1978; for a review, see Mysliweck, 1997). Moreover, pre-exposure to a conditioned stimulus (CS) facilitates conditioning in infant rats at an age when maternal cues are repeatedly presented during nursing bouts, whereas it retards conditioning in weanling and adult rats (Hoffmann and Spear, 1989) at ages when novel environmental cues are presented only rarely. Furthermore, when pups do learn, they appear to be less selective learners as compared to adult rats. That is, whereas an adult will focus on a narrow range of environmental stimuli in a learning situation, pups will learn associations with a broad range of such stimuli (Spear, Kutcharski, and Miller, 1989), thus promoting learned responses to multiple maternal features.

Clinical observations have taught us that attachment occurs not only to supportive caretakers, but to abusive caretakers as well. In fact, children tolerate considerable abuse while remaining strongly attached to the abusive caretaker (Helfer, Kemp, and Krugman, 1997). Initially, it seems counterproductive to form and maintain an attachment to an abusive caretaker; but from an evolutionary perspective, it may be better for an altricial infant to have a bad caretaker than no caretaker. We found that this aspect of human attachment can also be modeled in the infant rat. We and others have found that, during the first postnatal week, a surprisingly broad spectrum of stimuli can function as reinforcers to produce an odor preference in rat pups (Sullivan, Hofer, and Brake, 1986; Sullivan et al., 1986). As illustrated in figure 38.1, these stimuli range from apparently rewarding ones, such as milk and access to the mother (Alberts and May, 1984; Brake, 1981; Galef and Sherry, 1973; Johanson and Hall, 1979; Johanson and Teicher, 1980; Leon, 1973; McLean et al., 1993; Pedersen, Williams, and Blass, 1982; Weldon, Travis, and Kennedy, 1991; Wilson and Sullivan, 1994), to apparently aversive stimuli, such as moderate shock and tailpinch (Camp and Rudy, 1988; Spear 1978; Sullivan, Hofer, and Brake, 1986)—stimuli that elicit escape responses from the pups. It should be noted that threshold to shock (Stehouwer and Campbell, 1978) and behavioral response (Emerich et al., 1985) to shock does not change between the ages of 9 and 11 days. As pups mature and reach an age when leaving the nest becomes more likely (Bolles and Woods, 1965; pups begin to walk between PN9 and PN11), olfactory learning comes to resemble learning in adults more closely. Specifically, odor aversions are easily learned by 2-week olds and acquisition of odor preferences is limited to odors paired with stimuli of positive value (Camp and Rudy, 1988; Spear 1978; Sullivan and Wilson, 1995). Thus, the odor learning that underlies early attachment appears to take place in response to a very broad range of contingent events while pups are confined to the nest, but becomes more selective at a time in development when pups begin leaving the nest and encountering novel odors not associated with the mother.

Unique learning capabilities facilitating infant attachment appear throughout the animal kingdom and may have evolved to ensure that altricial animals easily form a repertoire of proximity-seeking behaviors, regardless of the specific qualities of the treatment they receive from the primary caretaker. Observations of mother-infant interactions within the rat nest demonstrate that maternal behaviors are sometimes painful to pups. For example, when the rat mother steps on pups upon entering/leaving the nest or when she retrieves pups by a leg rather than at the nape of the neck, broadband (mixed ultrasonic and audible range) vocalizations—the type associated with noxious stimuli—are elicited (White et al., 1992). It is certainly beneficial to pups not to learn an aversion to their mother's odor or inhibit approach responses to nest odors; instead, pups need to exhibit approach behaviors to procure the mother's milk, warmth, and protection. A similar phenomenon exists in avian species. Specifically, experiencing an aversive shock during exposure to an imprinting object strengthens the following response. For example, in a series of classic experiments by Hess (1962) and Salzen (1970), recently hatched chicks were shocked (3 mA for 0.5 s) while presented with a surrogate mother. The next day, chicks that were shocked showed a significantly stronger following response than chicks that were not shocked. With striking similarity to the infant rat, similar pairings in older chicks resulted in a subsequent aversion to the surrogate mother. Additional mammalian species in which similar phenomena have been documented include nonhuman primates (Harlow and Harlow, 1965), dogs (reviewed in Rajecik, Lamb, and Ohsneser, 1978), and humans (for a review, Helfer, Kempe, and Krugman, 1997).

In rats, early attachment-related odors appear to retain value into adulthood, although the role of the odor in modifying behavior appears to change with development. Work done independently in the labs of Celia
Moore (Moore, Jordan, and Wong, 1996) and Elliot Blass (Fillion and Blass, 1986) demonstrated that adult male rats exhibited enhanced sexual performance when exposed to the natural and artificial odors learned in infancy. These results support observations on the role of early experience on adult mate preference in other species, such as occurs in imprinting.

**Neural Plasticity Underlying Olfactory Preference Learning** The development of a specific olfactory-based attachment system in the rat pup during the first week and a half of life is associated with the acquisition of olfactory bulb neural changes. We found that rat pups express this modified olfactory bulb response to both natural maternal and artificial odors experienced in the nest (Sullivan et al., 1990), as well as to odors in controlled learning experiments (Johnson et al., 1995; Sullivan and Leon, 1986; Wilson and Sullivan, 1991; Wilson, Sullivan, and Leon, 1987). The modified olfactory bulb response is characterized by enhanced immediate-early gene activity (c-fos) and enhanced 2-deoxyglucose (2-DG) uptake in focal, odor-specific glomerular regions in response to the conditioned odor. Within the underlying neural substrate, modified single-unit response patterns of mitral/tufted cells near the enhanced glomerular foci were found (Wilson and Leon, 1988b; Wilson and Sullivan, 1990; Wilson, Sullivan, and Leon, 1987) and olfactory bulb anatomical changes reflected in enlarged glomeruli within these foci (Woo, Coopersmith, and Leon, 1987). As with the behavioral changes in attachment, these neural changes are retained into adulthood, but their acquisition is dependent upon experiences during infancy (Pager, 1974; Woo and Leon, 1988).

Many neurotransmitters have a role in early olfactory learning in neonatal rats—5-HT (McLean et al., 1993), DA (Barr and Wang, 1992; Weldon, Travis, and Kennedy, 1991), GABA (Okutani, Yagi, and Kaba, 1999), glutamate (Lincoln et al., 1988; Mickley et al., 1998), and opiates (Barr and Rossi, 1992; Kehoe and Blass, 1986). Roth and Sullivan (2000) have recently shown that PN8 pups treated with systemic naltrexone, a non-specific opioid antagonist, do not show a subsequent odor preference after odor-shock classical conditioning, suggesting an important role for opioids in early preference formation to noxious stimuli. However, the action of norepinephrine (NE) appears particularly important in neural plasticity during early development and in the form of olfactory (Brennen and Keverne, 1997; Wilson and Sullivan, 1994) and somatosensory (Landers and Sullivan, 1999b) learning-induced plasticity used in early attachment.

Norepinephrine input to the olfactory bulb is widespread throughout the granule cell layer as early as the...
first postnatal week (McLean and Shipley, 1991; Woo and Leon, 1995). The granule cell layer is composed of inhibitory interneurons modulating the output of the olfactory bulb through the adjacent mitral cells (Brunjes, Smith-Craft, and McCarty, 1985; Lauder and Bloom, 1974; Trombley and Shepherd, 1992; Wilson and Leon, 1988b). Wilson has shown that activation of the NE input to the infant rat’s olfactory bulb during an odor presentation maintains mitral cell responsiveness to that odor, preventing the habituation these cells normally exhibit to repeated odor presentations (Wilson and Sullivan, 1992). Subsequent work has shown that NE increases mitral cell responses to olfactory nerve input, suggesting that NE functions to increase the signal to noise ratio (Jiang et al., 1996).

The notion that this mechanism of plasticity underlies the neural and behavioral changes of the rapid, olfactory-based learning underlying rat pup attachment has received strong support from a variety of labs. First, work in Michael Leon’s lab, using microdialysis, showed that olfactory bulb NE increases during infant odor learning (Rangel and Leon, 1995). Second, recordings from olfactory bulb mitral cells during learning indicate that mitral cells maintain their responsiveness to odors in the experimental learning groups, but not control groups (Wilson and Sullivan, 1992). Third, infusion of NE into the olfactory bulb during an odor presentation is sufficient for pups to acquire an odor preference (Sullivan et al., submitted). Fourth, olfactory bulb NE (Langdon, Harley, and McLean, 1997; Sullivan et al., 1992) from the noradrenergic locus coeruleus (Sullivan et al., 1994) is necessary for infant rat learning. Thus, it is the contingent events of increasing olfactory bulb NE and odor stimulation that underlie the neural plasticity responsible for the acquisition of olfactory-based attachment behavior.

PARALLEL PROCESSES IN MATERNAL ATTACHMENT
Successful mother–infant interactions require the reciprocal responding of both individuals in the mother–infant dyad. Human mothers rapidly learn about their babies’ characteristics and can identify their babies’ cries, odors, and facial features within hours of birth (Eidelman and Katz, 1992; Katz et al., 1992; Porter, Gernoch, and McLaughlin, 1983). An animal model for this rapid learning has received considerable attention (for reviews, see Brennan and Keverne, 1997; Fleming et al., 1999). Indeed, there are interesting parallels between the early attachment behavior of infants and the attachment behavior of the newly parturient mother. In rats and sheep, a temporally restricted period of postpartum olfactory learning in the mother involving NE facilitates the mother’s learning about her young (Levy et al., 1990; Moffat, Suh, and Fleming, 1993; Pissounnier et al., 1985). It is possible that mammalian mothers and pups use similar neural circuitry to form their reciprocal attachment.

CLOSING THE SENSITIVE PERIOD FOR INFANTILE OLFACTORY PREFERENCE LEARNING. Olfactory bulb NE is not found in neurons intrinsic to the olfactory bulb, but arrives via direct projections from the noradrenergic pontine nucleus locus coeruleus (McLean and Shipley, 1991; Shipley, Halloran, and De la Torre, 1985). Recent evidence strongly suggests that developmental changes within the locus coeruleus may underlie the termination of the infant rat's unique learning abilities during the first week and a half of postnatal life. In neonatal rat pups, the locus coeruleus is not completely mature during the first week of postnatal life, nor is it an immature version of the adult locus coeruleus. Indeed, it has unique characteristics that result in an enhanced response of neonatal locus coeruleus neurons to sensory stimulation.

The infant locus coeruleus is more responsive to sensory stimuli than the adult locus coeruleus. Although the adult locus coeruleus is activated by sensory stimuli (Aston-Jones et al., 1994; Foote, Aston-Jones, and Bloom, 1980; Harley and Sara, 1992; Sara, Dyno-Laurent, and Herve, 1995; Vankov, Herve-Minvielle, and Sara, 1995), in comparison to the infant, it is less likely to respond to non-noxious stimuli (Kimura and Nakamura, 1985; Nakamura and Sakaguchi, 1990). Furthermore, the adult locus coeruleus habituates after repeated presentation of the stimuli (Vankov, Herve-Minvielle, and Sara, 1995) whereas the infant locus coeruleus fails to exhibit habituation (Kimura and Nakamura, 1985; Nakamura and Sakaguchi, 1990). Furthermore, a 1-second presentation of tactile stimulation is likely to cause a few millisecond response in the adult locus coeruleus, but a 20–30-second response in the first week infant locus coeruleus. Finally, the early infant locus coeruleus shows far more extensive electronic coupling through gap junctions, a process that appears to potentiate the locus coeruleus’s response (Marshall et al., 1991).

At about PN10, the infant locus coeruleus begins to take on adult characteristics (Marshall et al., 1991; Nakamura and Sakaguchi, 1990). These results suggest that the infant rat’s olfactory bulb receives unique input from the locus coeruleus during the first week of life and that maturational changes in the locus coeruleus during the second week, alter the properties of this input so as to bring to a close the early sensitive period for the imprinting-like learning underlying attachment in this species.

POSSIBLE ROLES OF OTHER BRAIN REGIONS IN EARLY ATTACHMENT. Although the simultaneous presenta-
Attachment, Emotion, and the Origins of Vocal Communication One of the best known responses to maternal separation is the infant's isolation call, a behavior that occurs in a wide variety of species (Lester and Boukydis, 1985; Newman, 1998). In the rat, this call is in the ultrasonic range (40 kHz) and appears in the first or second postnatal day (Noiriot, 1968). Pharmacological studies show that the ultrasonic vocalization (USV) response to isolation is attenuated or blocked in a dose-dependent manner by clinically effective anxiolytics that act at benzodiazepine and serotonin receptors; and conversely, USV rates are increased by compounds known to be anxiogenic in humans, such as benzodiazepine receptor inverse agonists (β-carbol ine, FG 1742) and GABA<sub>A</sub> receptor ligands such as pentylentetrazol (Hofer, 1996b; Miczek, Tornatsky, and Vivian, 1991). Within serotonin and opioid systems, receptor subtypes known to have opposing effects on experimental anxiety in adult rats also have opposing effects on infant calling rates. Neuroanatomical studies in infant rats show that stimulation of the periaqueductal gray area produces USV and chemical lesions of this area prevent calling (Goodwin and Barr, 1998). The more distal motor pathway is through the nucleus ambiguus and both laryngeal branches of the vagus nerve (Wetzel, Kelly, and Campbell, 1980). Higher centers known to be involved in cats and primates suggest a neural substrate for isolation calls involving primarily the hypothalamus, amygdala, thalamus, and hippocampus (Jurgens, 1994) — brain areas known to be involved in adult human and adult animal anxiety and defensive responses.

This evidence strongly suggests that social isolation produces an early affective state in rat pups that is expressed overtly in the rates of infant calling and covertly by the autonomic and adrenocortical systems. How does this calling behavior, and its inferred underlying affective state, develop as a communication system between mother and pup? Infant rat USVs are powerful stimuli for the lactating rat, capable of causing her to interrupt an ongoing nursing bout, initiate searching outside the nest, and direct her search toward the source of the calls (Smotherman et al., 1978). The mother's retrieval response to the pup's vocal signals then results in renewed contact between pup and mother. This contact in turn quiets the pup. The isolation and comfort responses in attachment theory are described as expressions of interruption and re-establishment of a social bond. Such a formulation would predict that since the pup recognizes its mother by her scent, pups made acutely anosmic would fail to show a comfort response. But anosmic pups show comfort responses that are virtually unaffected by loss of the capacity to recognize their mother in this way (Hofer and Shair, 1991). Instead, we and others have found multiple regulators of infant USV within the contact between mother and pup: warmth, tactile stimuli, and milk as well as her scent (Hofer, 1996b). Provision of these modalities separately, by experimental design, and then in combination elicits a graded response, with the maximum isolation calling rates occurring when all are withdrawn at once and with the full comfort response being elicited only when all are present at once.

From Physiological Regulation to Mental Representation The early learning processes and the widespread regulatory interactions (summarized in Table 38.1) are not involved in biological and behavioral development solely in rats; they are also the first experiences out of which human mental representations and their associated emotions arise. So far as we understand the process for the human, experiences made up of the infant's individual acts, parental responses, sensory impressions, and associated affects are laid down in memory during and after early parent-infant interactions (Gergeley and Watson, 1999; Stern 1985). These individual units of experience are integrated into something like a network of attributes in memory, invested with associated affect, and result in the formation of what Bowlby referred to as an "internal working model" of the relationship.

In the light of the research described in the previous sections, we can speculate that the early learning and maternal regulatory interactions described for rats occur also in human babies. For human infants, these experiences are gradually joined in memory and stored as the mental representations and related affective states that older children are able to describe in words. It seems likely that these mental structures combine the infant's newly developing capacities to anticipate events and respond to symbolic cues with the earlier biological functions of the "hidden" regulatory interactions through processes similar to the functional links involved in the classical conditioning of physiologic responses. In this way our concept of the "mental representation" can be thought to link together into a functional network within the child's brain the learned patterns of behavior and the physiological response systems previously regulated by the mother-infant interaction. In this way we can envisage the development of self-regulation of the behavioral and physiological systems underlying motivation and affect, gradually supplanting the sensorimotor, thermal, and nutrient regulatory systems found in the interactions of younger infants with their mothers. This would link biological systems with internal object representation and account
### Table 38.1
*Maternal regulators of infant systems in 2-week-old rat pups*

<table>
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<tr>
<th>Infant Systems</th>
<th>Maternal Regulators and Effects</th>
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</thead>
<tbody>
<tr>
<td>Behavioral</td>
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<tr>
<td>Activity level</td>
<td>Thermal—heat (+)</td>
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<tr>
<td></td>
<td>Thermal—cold (-)</td>
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<tr>
<td>Sucking:</td>
<td>Tactile stimulation (-)</td>
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<tr>
<td>Nutritive</td>
<td>Olfactory cues (-)</td>
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<td>Nonnutritive</td>
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<td>Vocalization ultrasound</td>
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<td>Autonomic</td>
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<tr>
<td>Sympathetic cardiac (β-adrenergic)</td>
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<td>Vasomotor (α-adrenergic)</td>
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<td>Endocrine</td>
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<td>Growth hormone</td>
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<td>ACTH, CRH</td>
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<td>Corticosterone</td>
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<td>Sleep–wake states</td>
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<td>Milk at gastric receptors (+)</td>
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<td></td>
<td>Milk at gastric receptors (-)</td>
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<td></td>
<td>Tactile stimulation (+)</td>
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<td>Tactile stimulation (-)</td>
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<td></td>
<td>Milk, acting on adrenal response to ACTH (-)</td>
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<td></td>
<td>Regularly timed tactile and/or nutrient stimuli</td>
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<td></td>
<td>REM duration (+)</td>
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<td>Awakening frequency (-); duration (-)</td>
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*The direction of regulation is indicated by (+) or (-) for up- or downregulation during normal ongoing mother–infant interaction. Upon maternal separation, release from regulation produces changes in the opposite direction in each infant system.*

for the remarkable upheaval of biological as well as psychological systems that take place in adult humans in response to cues signaling impending separation or in response to losses established simply upon hearing of a death, for example, by telephone (Hofer, 1984, 1996a).

Mother–infant relationships, which differ in quality and which necessarily involve different levels and patterns of behavioral and physiological regulation in a variety of systems, will be reflected in the nature of the mental representations present in different children as they grow up. The emotions aroused during early crying responses to separation, during the profound state changes associated with the prolonged loss of all maternal regulators, and during the reunion of a separated infant with its mother are apparently intense. These emotional states have commanded our attention; they are what everyone intuitively recognizes about attachment and separation, and what we feel about the people we are close to. As inner experiences, these occur at a different level of psychobiological organization than the changes in autonomic, endocrine, and neurophysiological systems we have been able to study in rats and monkeys, as well as in younger human infants. Hidden regulators thus form a developmental as well as a conceptual bridge between the tangible and the intangible in our understanding of attachment.

**Mother–infant interaction patterns, infant development, and maternal behavior in the next generation**

The actions of maternal regulators of infant biology and behavior are not limited to the mediation of responses to maternal separation. They exert their regulatory effects continuously, throughout the preweaning period and even beyond. A good illustration is the recent discovery of a major role for the mother–infant interaction in the development of the hypothalamic–adrenocortical axis (HPA) during the preweaning period. It has long been known that the rat from postnatal days 4 to 14, the pups' HPA response to isolation and mild stressors like saline injections are less intense than in the newborn or weaning periods; this stage is known as the "stress-hyporesponsive period" (Levine, 1994). Surprisingly, it has recently been found that this species-typical developmental stage is not the product of an intrinsic developmental program, but the result of hidden regulators at work within the ongoing mother–infant interaction. First, it was found that 9- to 12-day-old pups' basal corticosterone (CORT) level and the magnitude of the adrenocorticotropic hormone (ACTH) and CORT response to isolation was increased 5-fold after 24 hours of maternal separation (Stanton, Gutierrez, and Levine, 1988). Next, by utilizing our
concept of hidden regulators, Suchecki, Rosenfeld, and Levine (1993) attempted to prevent these separation-induced changes by supplying various components of the mother–infant interaction. They found that repeated stroking of the separated infants for as little as three 1-minute periods prevented the increase in ACTH response while providing milk by cheek cannula during separation prevented the separation-induced blunting of the adrenal CORT response to ACTH.

Thus the mother normally reduces infant HPA responses and separation eliminates this hyporesponsive state. Tracing these regulatory effects back to brain systems, Levine’s group has recently found that stroking (representing maternal licking) regulates the intensity of the immediate-early gene fos messenger RNA (mRNA) response in the paraventricular nucleus of the hypothalamus (PVN), and the corticotropin-releasing hormone (CRH) receptor mRNA expression as well, in PVN, amygdala, and other limbic system sites (Van Oers et al., 1998). It is intraorally administered milk, however, that regulates glucocorticoid receptor mRNA in the CA1 region of the hippocampus and CORT release from the adrenal in response to ACTH.

Through this anatomical and molecular neuromodulator analysis, Levine and colleagues discovered that maternal licking and milk delivery during sucking, independently exert a prolonged attenuating effect on the responsiveness of the HPA axis. This maternal regulatory effect, once established in the first few postnatal days, continues throughout most of the nursing period, finally declining as weaning occurs from day 15 to 21. These regulatory interactions achieve this effect by increasing the inhibitory feedback from hippocampal glucocorticoid receptors and by decreasing the hypothalamic stimulation of CRF and ACTH output. These regulatory effects on the pup’s brain can be rapidly reversed and stress responsiveness restored by maternal separation.

LONG-TERM EFFECTS  These preweaning studies on brain mechanisms underlying maternal regulation of HPA axis function suggested that qualitative differences in the patterns of early mother–infant interaction could have long-term effects on HPA responses to stress in adults similar to those we had discovered for adult blood pressure in spontaneously hypertensive rats (SHR) (Myers et al., 1989). Meaney and his colleagues used the maternal behavior observational approach developed in those studies to directly test this implication of the discovery of maternal regulators (Liu et al., 1997). They found that dams in their colony that were observed to have naturally high levels of licking, grooming, and of the high-arched back nursing position (LG/ABN) produced pups that were later found to be less fearful in a variety of behavioral tests and to show lower than normal HPA axis responsivity to restraint stress as adults than the offspring of dams that naturally showed the lowest levels of these early interactions.

In order to show that these differences in the offspring in adulthood were not simply reflections of a different genetic constitution in the two groups, Meaney and his colleagues handled daily the infants of the low LG/ABN group, an early intervention that is known to increase levels of the mother’s concurrent LG/ABN behaviors. By doing so, he showed that the adult behavior and HPA axis responses of the offspring also changed to closely resemble the patterns that naturally occurred in the previously studied high LG/ABN group.

These results showed that an intervention that alters the mother–infant interaction pattern also changes the adult fear behavior and physiological response characteristics of the offspring. One observation that currently occupies researchers in the attachment area is how mothers in one generation can pass on to their adult female offspring the attachment pattern they experienced as infants. The experiment I have just described provided a chance to find out if this transgenerational effect occurs in nonprimate species and to explore how it comes about. By allowing the offspring of the handled litters to rear another generation under normal laboratory conditions, Meaney and his colleagues (in press) went on to find that the mother–infant interaction established by these progeny resembled the one their mothers had been induced to have in their own infancy (high LG/ABN) rather than the one characteristic of their ancestors before the handling intervention (low LG/ABN) (Francis et al., 1999). Furthermore, the unmanipulated pups in these litters also went on to show the adult behaviors and hormonal stress patterns typical of offspring of high LG/ABN litters.

These experiments show the intergenerational transmission of mother–infant interaction patterns and the developmental effect of these interaction patterns on adult behavior and physiology. They provide an animal model for the neurobiological analysis of mechanisms underlying environmental effects on the transgenerational patterns of mothering and an experimental verification of one of the central tenets of present-day human attachment research.

Summary and perspective

We have re-examined the major propositions of attachment theory and described a series of component processes, discovered in experiments with laboratory rats,
that enable us to begin to understand the behavioral mechanisms involved and to identify their underlying neural substrates. We first described the processes that bring the neonatal mammal into close proximity to the mother, continue to keep the infant close, act to reunite the separated infant with the mother, and cause a complex patterned response to prolonged maternal separation. These several components tell us that an "enduring social bond" (to use Bowlby's term) has been formed, but we can now understand the bond in terms of separate processes that can be delineated as they work independently, serially, or in parallel to produce the familiar behavioral signs of "attachment." The discovery of these component processes allows us to begin to understand what makes up the "glue" that holds the infant to the mother. The discovery of regulatory interactions within the mother–infant relationship allows us to escape the circularity of the traditional attachment model, in which the response to separation is attributed to disruption of a social bond, the existence of which is inferred from the presence of that same separation response. Some of the individual processes described allow us to understand how the infant comes to identify and orient toward the mother by different means at different stages in development. Beginning before birth and continuing in the newborn period, novel processes of associative learning have been discovered that allow us for the first time to identify and understand the mysterious "imprinting-like process" that Bowlby envisioned as the altricial mammalian equivalent of avian imprinting. And, finally, we can begin to see how one of the consequences of these early learning processes, acting within repeated regulatory interactions, is to provide a novel source of experiences for the formation of the mental representation of the infant–mother relationship.

With this enlarged view, some processes have been discovered to play an important role in the development of proximity maintenance between infant and mother that were not previously considered to be important (e.g., prenatal learning). And events that were thought to be central to attachment have been found to be produced by other, independent mechanisms (e.g., separation responses). More broadly, our understanding of the evolutionary survival value of remaining close to the mother has been expanded to include the many pathways available for regulation of the infant's physiological and behavioral systems by its interactions with the caregiver. The relationship thus provides an opportunity for the mother to shape both the developing physiology and the behavior of her offspring, through her patterned interactions with the infant. Behavioral adaptations to environmental change occurring in the life of the mother can thus lead to "anticipatory" biological changes in the offspring—a novel evolutionary mechanism.

The discovery of regulatory interactions and the effects of their withdrawal allow us to understand not only the responses to separation in young organisms of limited cognitive-emotional capacity, but also the familiar experienced emotions and memories that can be verbally described to us by older children and adults. It is not that rat pups respond to loss of regulatory processes, while human infants respond to emotions of love, sadness, anger, and grief. Human infants, as they mature, can respond at the symbolic level as well as at the level of the behavioral and physiological processes of the regulatory interactions. The two levels appear to be organized as parallel and complementary response systems. Even adult humans continue to respond in important ways at the sensorimotor-physiologic level in their social interactions, separations and losses, continuing a process begun in infancy. A good example of this is the mutual regulation of menstrual synchrony among close female friends, an effect that takes place out of conscious awareness and has recently been found to be mediated at least in part by a pheromonal cue (Stern and McClintock, 1998). Other examples may well include the role of social interactions in entraining circadian physiological rhythms, the disorganizing effects of sensory deprivation, and the remarkable therapeutic effects of social support on the course of medical illness (reviewed in Hofer, 1984). In this way, adult love, grief, and bereavement may well contain elements of the simpler regulatory processes that we can clearly see in the attachment responses of infant animals to separation from their social companions.

This is perhaps the most challenging area for future research: to find out how to apply what we have learned in basic brain and behavior studies to the human condition. Studies on other animals cannot be used to define human nature, but many of the principles and new ways to approach the mother–infant interaction described in this chapter can be useful in studies of the human mother–infant relationship. We must take into account obvious differences between species, such as the primacy of olfaction and tactile senses in the newborn rat as contrasted to the wider range of sensory available to the human newborn. For example, the learning processes regulating approach and proximity that are based on olfaction and tactile senses in the rat pup are likely to be mediated by visual and auditory systems as well as olfaction and touch in the human newborn.

At present, there is widespread use of the concept of regulation as inherent in the mother–infant interaction in humans. This word is generally used in two ways. First, it is used to refer to the graded effects of different patterns of interaction on the emotional responses of
the infant, the so-called "regulation of affect" (Schorc 1994). Second, it is used to refer to how the behaviors initiated by the infant or mother and/or their responses to each other act to regulate the interaction itself, its tempo or rhythm (hence its "quality"), or the distance (both psychological and physical) between the members of the dyad (Gergeley and Watson, 1999). The word "regulation" is used extensively in the literature on molecular genetic, cellular, and electrophysiological brain processes so that it serves as a useful conceptual link across wide differences in the level of organization at which developmental processes are studied.

A role for nonverbal features of the early mother-infant interaction in the specification of lasting mental representations of maternal behavior in the adult is a central hypothesis of clinical attachment theory. It would be difficult to confirm clinically this useful idea with any degree of certainty. But the transgenerational effects on maternal behavior and the HPA axis described in this chapter can serve as a research model for understanding the psychobiological mechanisms for this important effect. Prospective clinical studies from infancy to childhood would be most interesting and could reveal which residues of particular early interactions can be related to which later characteristics of the stories, play, or social relationships of older children, and eventually the parental behavior of adults.

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