

II

Primal Connections

2 The Biochemistry of Family Commitment and Youth Competence: Lessons from Animal Models

Larry J. Young

Emory University School of Medicine

Darlene D. Francis

University of California, Berkeley

Recent advances in neuroscience are beginning to provide fresh insights into the mechanisms for social interaction, including the molecular and cellular processes involved in the formation of social bonds. Understanding the biochemical factors that contribute to social attachment, as well as the biological consequences of parent–offspring interactions, may contribute to better understanding of normative development. In this chapter, we will review some of the results from studies of nonhuman animals revealing the neurobiological causes and consequences of social bonding and parental care. First, we describe a monogamous rodent that has provided an extensive understanding of the molecular and cellular basis of selective social attachment between mates. This research may serve as a model for understanding social bonding in general as the same mechanisms may contribute to many aspects of social attachments. We will then provide an overview of the underlying neurobiology of parental care. Finally, we will describe studies that examine the enduring consequences of differing parental styles on behavior and neurochemistry and provide a model of how these behaviors may be perpetuated across generations. We must stress that the reader bear in mind that most of the data presented here are from rodent studies, and there is very little evidence at this time that these same underlying mechanisms are similar in humans. However, understanding these processes in animals provides a perspective for thinking about how biological and environmental processes may govern behavioral outcomes in human beings.

Biochemistry of Family Commitment and Attachment in Rodents

The prototype human nuclear family structure consists of a mother, a father, and offspring. Thus, family commitment consists of, first, the bond between the parents, and then, between the parents and the offspring. In contrast with our species, parental care in most mammals is provided entirely by a single parent, the mother. Roughly 5% of mammalian species are socially monogamous, however, with family units consisting of both parents and offspring. One monogamous species, the prairie vole, has been useful in deciphering the neurobiological basis of adult social bonds between mates (Carter, 1998; Insel & Young, 2001). Remarkably, it appears that similar processes that underlie the attachments between mates may also contribute to parental care.

Social Attachment in Adult Voles

Prairie voles (*Microtus ochrogaster*) have become one of the most useful animal models for investigating the biochemistry of social attachment (Insel & Young, 2001; Young, Lim, Gingrich, & Insel, 2001). Prairie voles are hamster-sized rodents found in the Midwestern prairie of the United States. Studies in the field indicate that prairie voles form lifelong bonds with their mates and produce multiple litters together (Carter & Getz, 1993). Prairie vole nests typically consist of a mating pair and multiple generations of offspring, which contribute to the parenting of the youngest litter. The male prairie vole defends the nest and contributes significantly to the rearing of the offspring. Over the past 10 years, a series of laboratory studies has begun to uncover the neurochemical events that cement the bond between the parent voles.

The pair bonding process in voles can be easily studied in the laboratory, making it amenable to pharmacological manipulations. In the lab, pair bonding is assessed using a partner preference paradigm (Figure 1). In this procedure,

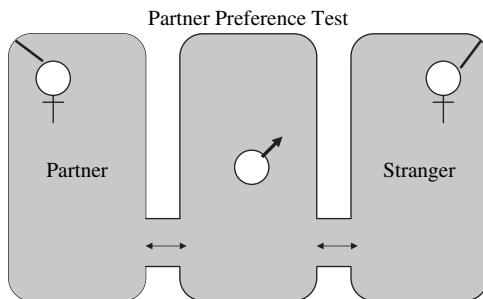


Figure 1. Schematic of the partner preference testing arena used to test for the development of a social bond between two adult voles. In the example, the male is being tested for a social attachment

an adult male and female prairie vole are paired, during which experimental manipulations (i.e., duration of cohabitation, presence or absence of mating, pharmacological treatments, etc.) are performed. After the designated time of cohabitation, the pair is separated and then placed in a partner preference testing arena. The arena consists of three chambers: the “partner” chamber, in which the partner is tethered to restrict its movement; the “stranger” chamber, in which a stimulus animal of equal stimulus value as the partner is tethered; and the neutral chamber, which is connected to the other two chambers by means of tubing. The experimental animal is placed in the neutral chamber and is allowed to roam freely between each chamber for a designated amount of time, typically 3 hours. An animal is said to have developed a partner preference if it spends more than twice as much time in contact with its partner than with the novel female.

Using the partner preference paradigm, some of the parameters of pair bond formation have been examined. The data suggests that while partner preferences can form after long periods of cohabitation, mating facilitates partner preference formation in both male and female prairie voles. For example, in one study, 6 hours of cohabitation without mating was not sufficient for the female to develop a partner preference, whereas the same duration of cohabitation with mating was sufficient (Williams, Catania, & Carter, 1992). Similar results have been obtained with males (Insel, Preston, & Winslow, 1995). Thus, it appears that both the quality and quantity of social interactions during the cohabitation contribute to the formation of the pair bond. Mating, while not critical, likely acts to strengthen the pair bond in the monogamous vole. Using the partner preference paradigm in conjunction with pharmacological manipulations, we have begun to understand the chemical triggers and neural circuits underlying this process.

What biochemical processes underlie the pair bonding process? As mentioned earlier, social attachment is fairly rare between adults in mammals, although strong attachments between mother and their offspring are widespread. It is conceivable that similar neural and biochemical systems that are involved in regulating mother–infant relationships have been co-opted to produce the pair bond. This, in fact, appears to be the case. Oxytocin (OT) is a neuropeptide hormone produced in the hypothalamus, which sends projec-

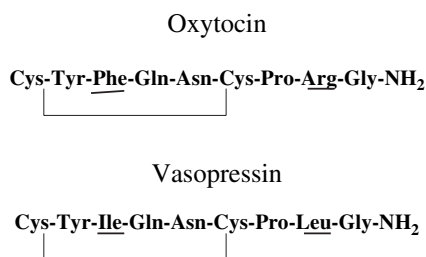


Figure 2. The molecular structures of oxytocin and vasopressin. Note that these peptides differ only at two amino acid residues (underlined)

tions to the posterior pituitary as well as into the brain (Figure 2) (Gainer & Wray, 1994). OT is released into the plasma from the posterior pituitary during labor and is thought to play a role in facilitating parturition through its actions on OT receptors in the uterus. OT is also released into the plasma in a pulsatile manner during nursing where it stimulates the milk ejection reflex, making breast-feeding possible. As will be discussed later in this chapter, studies in both rodents and sheep have suggested that OT released within the brain also plays an important role in initiating maternal behavior as well as facilitating the selective bond between mother and offspring. Several studies have now demonstrated that this same neuropeptide plays a role in the development of the pair bond in the female prairie vole.

Injections directly into the female prairie vole brain of a drug that blocks OT action, prior to cohabitation and mating, inhibits the development of a partner preference (Insel & Hulihan, 1995). Furthermore, infusion of OT into the female prairie vole brain facilitates the formation of the partner preference in the absence of mating (Williams, Insel, Harbaugh, & Carter, 1994). How can a molecule such as OT facilitate a phenomenon as complex as a social bond? First, we must understand how OT exerts its biological action. When released into the brain, OT acts as a neuromodulator, affecting neuronal function and communication. It does this by acting on receptors located in distinct areas of the brain, each of which has a unique function, such as altering perception, motivation, or emotion. Receptors are the molecules on the target cells that transduce the signal of the peptide hormone. Therefore, the first step in understanding the neurobiological mechanisms by which OT might facilitate a pair bond is to determine the location of the receptors in the brain. As it turns out, OT receptors are located in several brain regions known to affect emotionality, including the septum, amygdala, hypothalamus, nucleus accumbens, and prelimbic cortex (Insel & Shapiro, 1992). The first clues as to which particular brain sites are critical for social attachments in voles came from a comparative study in which locations of OT receptors in the prairie vole and that of the montane vole were compared (Insel & Shapiro, 1992). Montane voles, inhabitants of the Rocky Mountain region, are very closely related and similar in appearance to prairie voles, but have a very different family structure (Jannett, 1980). Montane voles do not form pair bonds, and female montane voles raise pups alone until they abandon them after only 2 weeks. The difference in OT receptor distribution in the brain of these closely related species is remarkable. Among other differences, there is a striking difference in OT receptors in the so-called reward circuitry of the brain; the nucleus accumbens and prelimbic cortex. Whereas montane voles have few OT receptors in these regions, prairie voles have high densities of receptors there (Figure 3).

Pharmacological studies have now demonstrated that the activation of OT receptors in these particular sites is critical for partner preference formation. Site-specific injections of an OT receptor blocker into the nucleus accumbens or prelimbic cortex completely disrupt the ability of a female prairie vole to bond with her mate (Young et al., 2001).

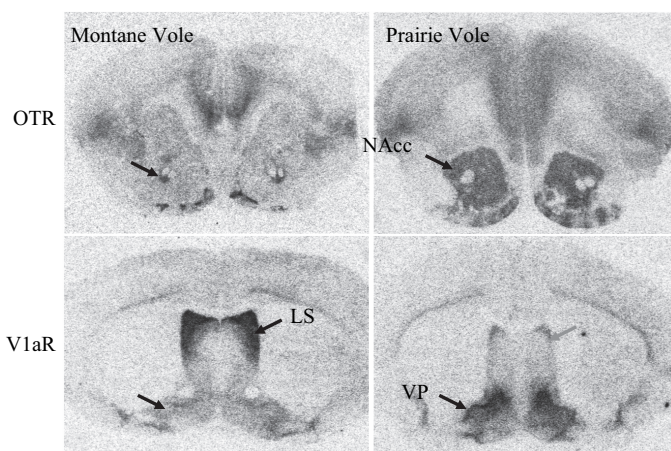


Figure 3. Distribution of oxytocin receptor (OTR; upper) and vasopressin receptor (V1aR; lower) binding in the montane (left) and prairie (right) vole brain. Note the high levels of receptors in the nucleus accumbens (NAcc) and ventral pallidum (VP) in the monogamous prairie vole compared with the montane vole. LS, lateral septum

In a parallel series of studies, it has become clear that when it comes to male prairie voles, a related neuropeptide, arginine vasopressin (AVP), is playing a larger role in the pair bonding process (Winslow, Hastings, Carter, Harbaugh, & Insel, 1993), although OT may also contribute to the process (Cho, DeVries, Williams, & Carter, 1999). AVP is structurally related to OT, a cyclical nonapeptide differing from OT in only two amino acid positions (Figure 2). Like OT, AVP is also synthesized in the hypothalamus and transported to the posterior pituitary (Gainer & Wray, 1994). However, extrahypothalamic AVP neurons from the amygdala project into the forebrain, where they are thought to influence behavior through interactions with the V1a subtype AVP receptor (V1aR). These extrahypothalamic AVP projections are sexually dimorphic, with males producing far more AVP than females (DeVries, 1990). Infusion of a compound that blocks V1aR activation prior to cohabitation and mating prevents male prairie voles from displaying a partner preference (Winslow et al., 1993). Conversely, infusions of AVP during an abbreviated cohabitation without mating facilitate the formation of a partner preference.

How might AVP be facilitating pair bond formation in the male prairie vole? In a study parallel to that described above for OT, the locations of V1aR in the brains of the prairie and montane voles were compared, and several intriguing differences were found (Insel, Wang, & Ferris, 1994). One difference of particular interest is in the ventral pallidum (Figure 3) (Young et al., 2001), which is located very closely to, and is reciprocally connected to, the nucleus accumbens. Like the nucleus accumbens, the ventral pallidum is part of the reward circuitry in the brain (McBride, Murphy, & Ikemoto, 1999). As it turns out, in several unrelated monogamous species, V1aR is abundant in the ventral

pallidum compared with nonmonogamous related species. A recent study suggests that the density of V1aR in the ventral pallidum is directly related to the ability to form a pair bond. In this study, viral vector-mediated gene transfer was used to increase the density of V1aR in the ventral pallidum of male prairie voles (Pitkow, Sharer, Ren, Terwilliger, & Young, 2001). Compared with control males injected with an inactive gene, males with increased ventral pallidal receptor density were much more likely to form a partner preference and displayed increased affiliative behavior toward juvenile prairie voles.

It seems that in male and female prairie voles, two closely related molecules facilitate social attachment by activating two separate components of the same reward circuit, namely, the nucleus accumbens for OT and the ventral pallidum for AVP. These regions are rich in another neurotransmitter, dopamine, which has widely been associated with reward. Drugs of abuse, such as amphetamines and cocaine, are thought to produce their euphoric effects by modulating the dopamine system in these regions (McBride et al., 1999). In fact, injections of cocaine into these regions of the rat brain result in the development of a conditioned *place* preference (Gong, Neill, & Justice, 1996). That is, the rat prefers to be in the environment in which it once received an injection of drug into these regions. Perhaps the activation of the OT and AVP receptors in these regions during mating results in the development of a conditioned partner preference in prairie voles. Because montane voles have few receptors in these regions, mating and/or the release of peptide in the brain would not result in the formation of a partner preference but may instead elicit other types of behaviors. If true, social attachment may be mediated, at least in part, by the brain's natural reward systems, which are so often abused by the use of illicit drugs. In addition to facilitating social attachment, both OT and AVP appear to increase nonspecific social interaction, or affiliative behavior (Cho et al., 1999; Witt, Winslow, & Insel, 1992; Young, Nilsen, Waymire, MacGregor, & Insel 1999), as well as social recognition (Englemann & Landgraf, 1994; Ferguson, Young, Hearn, Insel, & Winslow, 2000). Thus, it appears that these molecules may play an important role in modulating many aspects of social relations beyond selective bonding.

There are several caveats to the proposed mechanisms underlying attachment described thus far. First, these data in no way imply that OT and AVP are the only biochemicals involved in social attachment. There is likely a multitude of other factors, dopamine, for instance, that are involved in the chain of events leading to social bonding. Likewise, the reward circuitry is likely not the only brain sites involved in OT- or AVP-facilitated attachments. Other regions, such as the amygdala, hypothalamus, and lateral septum, likely also contribute in the cascade of events that lead to the social bond. Finally, as stated in the outset, one must be careful when making conjecture on the neural basis of human behavior based on rodent studies. Clearly, these neuropeptides or circuitry may be involved in human bonding, but there are very little data at this point, as experimental manipulation of human bonding cannot be performed in the laboratory. However, it should be noted that OT and AVP

are released into the plasma during sexual activity in humans. It is plausible that these hormones released during intimate moments serve to strengthen psychological bond between partners.

Biochemistry of Parental Commitment in Mammals

Maternal Responsiveness

In many species, females that have not produced offspring themselves are either indifferent to or avoid young. Then, after delivery of their own young, they become obsessed with caring for them. This nurturing motivation remains strong until the offspring leave the care of their mother. What biochemical changes during pregnancy and delivery promote maternal care? The laboratory rat has provided a great deal of insight into this process. Maternal behavior in rats consists of four basic behaviors: nest building, pup retrieval, nursing, and licking and grooming, each of which emerges around the time of parturition (Figure 4). One of the classic studies of behavioral neuroscience demonstrated that molecules circulating in the blood during pregnancy facilitate the onset of maternal care. In this study, a virgin rat was continuously transfused with the blood of a pregnant rat, such that hormones secreted by the pregnant rat reached the brain of the virgin (Terkel & Rosenblatt, 1972). The virgin rat became maternal precisely coincidental with the onset of parental care of the parturient rat. Several studies have subsequently identified several hormonal molecules that promote maternal responsiveness (Young & Insel, 2002).

As it turns out, many of the same hormones secreted in the body to promote the development and delivery of young also act in the brain to facilitate the nurturing behavior of the mother. First, steroid hormones, such as estrogen and progesterone, are secreted at high levels during pregnancy. Among other things, these hormones serve to maintain the integrity of the uterus to support the implanted embryos. Several studies have now shown that a virgin female rat can be primed to display maternal behavior simply by injecting her with steroid hormones in such a way as to mimic the natural changes of these molecules during pregnancy and labor. Injections of estrogen and progesterone, followed by progesterone withdrawal, facilitate the onset of maternal behavior (Bridges, 1984). These hormones appear to be acting in the preoptic area of the hypothalamus, a brain region that has a high density of estrogen and progesterone receptors and that has been implicated in the expression of maternal behavior. However, these hormones are not acting alone to produce the natural switch toward nurturing behavior, as female rats treated in this way do not display immediate maternal care but require several hours of pup exposure before pup retrieval is observed.

Two neuropeptide hormones, prolactin and OT, appear to be critical for the induction of parental care in rats. Prolactin is a hormone released into the plasma in large amounts from the anterior pituitary just after parturition. As

its name suggests, this hormone is involved in the regulation of lactation. Thus, it is not surprising that it also acts as a trigger to facilitate maternal behavior. Drugs that block prolactin release prevent the onset of parental care (Bridges, 1984), and infusions of prolactin into the lateral ventricles of the brain (Bridges, Numan, Ronsheim, Mann, & Lupini, 1990) or directly into the preoptic area facilitate maternal behavior (Bridges et al., 1997). Furthermore, mice with a genetic mutation in the prolactin receptor completely neglect their newborn offspring (Lucas, Ormandy, Binart, Bridges, & Kelly, 1998).

OT, which, as mentioned earlier, promotes uterine contractions and stimulates milk ejection during nursing, also facilitates the onset of maternal behavior in virgin rats within minutes after injection (Pedersen & Prange, 1979). Furthermore, blockade of OT receptors in the medial preoptic area and the ventral tegmental area prevents the onset of parental care in postpartum dams (Pedersen, Caldwell, Walker, Ayers, & Mason, 1994). Interestingly, OT is not required for the maintenance of maternal behavior once it has been established, as blocking OT receptors or destroying the brain's source of OT does not abolish it (Insel & Harbaugh, 1989). In addition to the direct effect on the initiation of maternal behavior, OT probably modulates maternal-offspring interactions through its anxiolytic effects. OT likely produces its anxiolytic



Figure 4. Maternal behavior in the rat consists of nest building (upper left), pup retrieval (upper right), nursing (lower left), and licking and grooming (lower right)

effects through its interactions with receptors in the central nucleus of the amygdala (Bale, Davis, Auger, Dorsa, & McCarthy, 2001).

Rodents typically display promiscuous maternal behavior. That is, they do not discriminate between their own pups and those from other females. Sheep, on the other hand, are selectively maternal and form strong bonds with their own lambs within minutes after they give birth (Kendrick et al., 1997). A series of studies have demonstrated that OT plays a critical role in forming that bond between the ewe and her lamb. For example, injections of OT into the brain of a steroid-primed ewe will cause her to accept a strange lamb as her own (Kendrick, Keverne, & Baldwin, 1987).

Paternal Care

Whereas common laboratory rats have provided most of our knowledge of the neurochemistry of maternal responsiveness, they are useless for examining the biology of paternal care. Again, the monogamous prairie voles have provided some insight. As discussed earlier, AVP plays an important role in facilitating social attachments between mates in male prairie voles. One study has demonstrated that this neuropeptide facilitates paternal care as well (Wang, Ferris, & DeVries, 1994). Infusion of AVP directly into the lateral septum of prairie vole males results in an increase in pup retrieval and time in contact with the pups.

Some studies suggest that prolactin may be involved in paternal care as well. Measurements of prolactin levels in the plasma of expecting father marmosets, which display biparental care, suggested that prolactin levels increase concurrently with the onset of paternal care (Dixon & George, 1982). Furthermore, prolactin levels are elevated in both male and female parentally inexperienced marmosets after carrying infants.

Together, the studies on social attachment and parental care indicate that the peptide hormones OT and AVP play a prominent role in family investment in rodents. Interestingly, species with different family structures display different patterns of receptor localization in the brain. Thus, factors that influence the OT and AVP systems will likely lead to perturbations in social behaviors. As will be discussed in the following section, early life experiences, such as the pattern of parental behavior one experiences during development, alter these neuropeptide systems, thereby possibly providing a biochemical mechanism by which adolescent experience alters adult social competence.

Developmental Influences on Youth Competence in Rodents

It has been known for more than 40 years that neonatal experiences in rats have a powerful effect on emotional reactivity of the offspring. An early handling (infantile stimulation) paradigm was shown to result in reduced

emotional reactivity, and a prolonged maternal separation paradigm was found to confer increased emotional reactivity in rats (Plotsky & Meaney, 1993). For example, early handling decreases and prolonged maternal separation increases hypothalamic–pituitary–adrenal activation in response to restraint stress or mild foot shock stress (Ladd, Owens, & Nemeroff, 1996; Liu, Caldji, Sharma, Plotsky, & Meaney, 2000; Plotsky & Meaney, 1993). Not only are there permanent changes in behavioral responses to stressful situations, the underlying neural systems believed to mediate these behaviors are also changed. For example, rats that received more maternal stimulation as pups have altered levels of stress hormone receptors (glucocorticoid receptor) in the hippocampus, a brain region that plays a central role in the regulation of the stress response (Meaney et al., 1996).

Recent studies have begun to examine the mechanisms by which early experience confers these long-term effects. In an important study from Michael Meaney's group at McGill University, researchers discovered that the mothers of handled rat pups showed increased amounts of maternal attention in the form of pup licking compared with mothers of nonhandled pups (Liu et al., 1997). Moreover, there are natural individual differences in the amount of licking that a mother rat spontaneously exhibits toward her pups, and these individual differences in "maternal style" are consistent across litters. More interestingly, these differences in the quality of maternal care translated into differences in stress reactivity and anxiety levels in the offspring (Caldji, Tannenbaum, Sharma, Francis, & Plotsky, 1998). The offspring of mothers with high levels of licking exhibit reduced emotionality and stress responsiveness relative to the offspring of low-licking mothers (Caldji et al., 1998; Liu et al., 1997). Not only do these differences in maternal attention predict emotionality of the offspring, they also predict how the offspring will mother their own pups. That is, the offspring of high-licking mothers also showed high levels of licking toward their own pups in adulthood (Francis, Diorio, Liu, & Meaney, 1999). One important question to ask is whether this variability in maternal care is genetically transmitted to the offspring or somehow "acquired" by the pups. To answer this question, pups from low-licking mothers were cross-fostered to high-licking mothers. In adulthood, these cross-fostered females showed reduced emotional reactivity and anxiety when placed in stressful situations and high levels of licking toward their own offspring (Figure 5).

These various studies of individual differences in maternal style reveal that even natural variations in mothering can result in profound differences not only in the offspring's emotional reactivity but also in the offspring's behavior toward her own infants. This suggests a nongenomic mechanism for the transmission of behavioral traits. Remarkably, these differences in behavioral state are associated with reliable differences in brain chemistry, specifically oxytocin. Females that have received higher levels of maternal stimulation as infants have higher concentrations of oxytocin receptors specifically in the amygdala. Males do not show this difference but have increased levels of vasopressin receptors in the same region (Francis, Young, Meaney, & Insel, 2002).

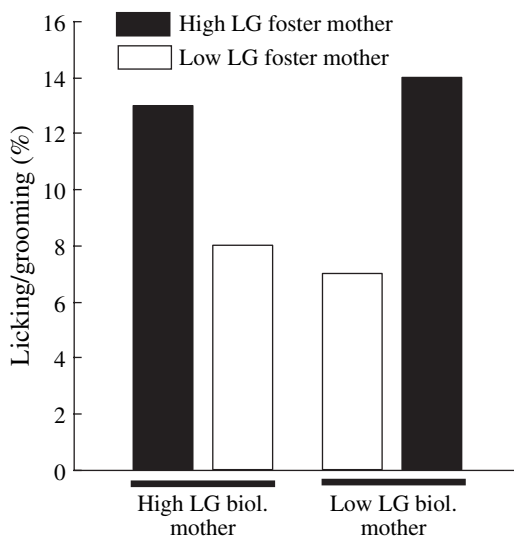


Figure 5. Nongenomic transmission across generations of maternal behavior patterns. Values represent the relative frequency of licking and grooming of pups for mothers that were fostered as pups to either high-licking-grooming (LG) or low-licking-grooming mothers. Pups reared by high-licking-grooming mothers exhibit high levels of licking and grooming as mothers regardless of the behavior of their biological mothers. (Data from Francis, Diorio, Liu, & Meaney, 1999; 286:1155–1158)

These findings suggest that differences in maternal care result in specific changes in the very systems implicated in social bond formation. At a molecular level, it appears that the kind of mothering a rat receives determines the development of the neurochemical systems critical for parental care and social interaction. Although we do not know the mechanisms by which maternal licking determines these neurochemical differences, Champagne, Diorio, Sharma, and Meaney (2001) have discovered an important role for estrogen receptors in this process. They have demonstrated that estrogen administration is effective in promoting maternal responsivity in virgin female rats *only* in the offspring of high-licking mothers. Estrogen was completely ineffective in altering maternal responsivity in offspring of low-licking females. The increase in maternal responsivity in high-licked females was also positively correlated with an increase in OT receptor expression in several maternal brain sites. These results demonstrate a strong interaction between the OT and estrogen systems, and how in concert, they may serve to induce and promote the expression of maternal care.

The studies described thus far are in essence investigating some very old and popular concepts regarding maternal and parental care. We begin to understand how phrases such as “Like mother, like daughter” and “Like begets like” may indeed have very biologically relevant roots. As we have seen, the quality of maternal care one receives regulates the very genes that modulate both coping with stressful stimuli and the quality of social interaction and nurturing provided to one’s own offspring (Figure 6). Without interruption,

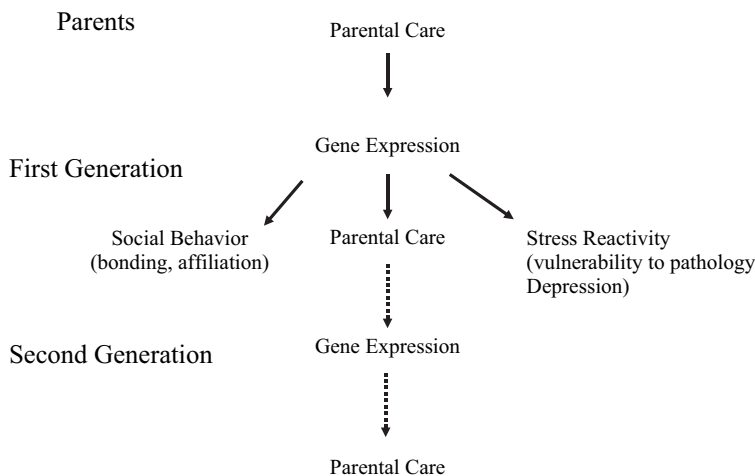


Figure 6. Figure demonstrating early environmental–parental regulation of genes in offspring, which subsequently regulate behavior, stress reactivity, and parental care in the following generation of animals. It is easy to observe how this cycle of transmission can occur *ad infinitum* without an environmental intervention to disrupt it

the parenting and coping styles are transmitted from generation to generation through this modulation of the brain's biochemistry. Whereas scientists have known for decades about the relationship between quality of early life environments and development, we have only fairly recently begun to address these very “human” questions concerning social bonding and parental care using animal models. Similar to results obtained using rodent and primate models of differing early-life events, it appears as if similar processes may indeed be at work in humans.

Relevancy to Humans

Two human studies investigating the quality of early-life events in relationship to adult health and well-being demonstrate how successful and effective animal models have been at modeling the human condition. Russek and Schwartz, in 1997, published a follow-up study to an experiment conducted in the early 1950s at Harvard University that investigated the reflections of a sample of healthy, undergraduate Harvard men about their feelings of warmth and closeness to their parents. When these same men were interviewed 35 years later and their medical and psychological histories were taken, a dramatic relationship emerged. Of those men who did not perceive themselves to have had a warm relationship with their parents, 91% had diagnosed diseases (such as coronary artery disease, hypertension, duodenal ulcer, alcoholism), when interviewed. Men who perceived warm relationships with their parents demonstrated only a 45% incidence of disease. This very

simple follow-up study reinforces the wealth of animal data that demonstrate that the quality of an organism's early life and environment may play a profound role in the life of that animal later in adulthood

Another more recent study published in the *Journal of the American Medical Association* was the first human report to demonstrate lasting changes in stress reactivity in adult women who had suffered trauma early in life (Heim et al., 2000). The study was modeled after many of the laboratory animal studies described earlier in this chapter. The authors demonstrated that women subjected to early-life stress such as childhood physical or sexual abuse had as adults increased physiologic and hormonal responses to stress. They hypothesized that this increased reactivity may subsequently increase vulnerability to develop stress-related psychiatric disorders later in adulthood.

The results from these two studies suggest that processes at work in voles, rats, and other furry mammals may indeed be directly relevant to humans, underscoring the importance of basic animal models to the study of complex behaviors, including attachment and parental care.

References

- Bale, T. L., Davis, A. M., Auger, A. P., Dorsa, D. M., & McCarthy, M. M. (2001). CNS region-specific oxytocin receptor expression: Importance in regulation of anxiety and sex behavior. *Journal of Neuroscience*, 21, 2546–2552.
- Bridges, R. S. (1984). A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat. *Endocrinology*, 114, 930–940.
- Bridges, R. S., Numan, M., Ronsheim, P. M., Mann, P. E., & Lupini, C. E. (1990). Central prolactin infusions stimulate maternal behavior in steroid-treated, nulliparous female rats. *Proceedings of the National Academy of Sciences USA*, 87, 8003–8007.
- Bridges, R. S., Robertson, M. C., Shiu, R. P. C., Sturgis, J. J., Henriquez, B. M., & Mann, P. E. (1997). Central lactogenic regulation of maternal behavior in rats: Steroid dependence, hormone specificity, and behavioral potencies of rat prolactin and rat placental lactogen I. *Endocrinology*, 138, 756–763.
- Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., & Plotsky, P. M. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of behavioral fearfulness in adulthood in the rat. *Proceedings of the National Academy of Sciences USA*, 95, 5335–5340.
- Carter, C. S. (1998). Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology*, 23, 779–818.
- Carter, C. S., & Getz, L. L. (1993). Monogamy and the prairie vole. *Scientific American*, 268, 100–106.
- Champagne, F., Diorio, J., Sharma, S., & Meaney, M. J. (2001). Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proceedings of the National Academy of Sciences USA*, 98, 12736–12741.
- Cho, M. M., DeVries, A. C., Williams, J. R., & Carter, C. S. (1999). The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (*Microtus ochrogaster*). *Behavioral Neuroscience*, 113, 1071–1079.
- DeVries, G. J. (1990). Sex differences in the brain. *Journal of Neuroendocrinology*, 2, 1–13.
- Dixon, A. F., & George, L. (1982). Prolactin and parental behaviour in a male New World primate. *Nature*, 299, 551–553.
- Englemann, M., & Landgraf, R. (1994). Microdialysis administration of vasopressin into the septum improves social recognition in Brattleboro rats. *Physiology and Behavior*, 55, 145–149.

- Ferguson, J. N., Young, L. J., Hearn, E. F., Insel, T. R., & Winslow, J. T. (2000). Social amnesia in mice lacking the oxytocin gene. *Nature Genetics*, 25, 284–288.
- Francis, D., Diorio, J., Liu, D., & Meaney, M. J. (1999). Variations in maternal care form the basis for a non-genomic mechanism of inter-generational transmission of individual differences in behavioral and endocrine responses to stress. *Science*, 286, 1155–1158.
- Francis, D. D., Young, L. J., Meaney, M. J., & Insel, T. R. (2002). Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: Gender differences. *Journal of Neuroendocrinology*, 14, 349–353.
- Gainer, H., & Wray, W. (1994). Cellular and molecular biology of oxytocin and vasopressin. In E. Knobil & J. D. Neill (Eds.), *The physiology of reproduction* (pp. 1099–1129). New York: Raven Press.
- Gong, W., Neill, D., & Justice, J. B. (1996). Conditioned place preference and locomotor activation produced by injection of psychostimulants in ventral pallidum. *Brain Research*, 707, 64–74.
- Heim, C., Newport, J., Heit, S., Graham, Y. P., Wilcox, M., Bonsall, R., et al. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *Journal of the American Medical Association*, 284, 592–597.
- Insel, T. R., & Harbaugh, C. R. (1989). Lesions of the hypothalamic paraventricular nucleus disrupt the initiation of maternal behavior. *Physiology and Behavior*, 45, 1033–1041.
- Insel, T. R., & Hulihan, T. (1995). A gender-specific mechanism for pair bonding: Oxytocin and partner preference formation in monogamous voles. *Behavioral Neuroscience*, 109, 782–789.
- Insel, T. R., Preston, S., & Winslow, J. T. (1995). Mating in the monogamous male: Behavioral consequences. *Physiology and Behavior*, 57, 615–627.
- Insel, T. R., & Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proceedings of the National Academy of Sciences USA*, 89, 5981–5985.
- Insel, T. R., Wang, Z., & Ferris, C. F. (1994). Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. *Journal of Neuroscience*, 14, 5381–5392.
- Insel, T. R., & Young, L. J. (2001). Neurobiology of social attachment. *Nature Neuroscience*, 2, 129–136.
- Jannett, F. J. (1980). Social dynamics of the montane vole *Microtus montanus*, as a paradigm. *Biologist*, 62, 3–19.
- Kendrick, K. M., Costa, A. P. C. D., Broad, K. D., Ohkura, S., Guevara, R., Levy, F., et al. (1997). Neural control of maternal behavior and olfactory recognition of offspring. *Brain Research Bulletin*, 44, 383–395.
- Kendrick, K. M., Keverne, E. B., & Baldwin, B. A. (1987). Intracerebroventricular oxytocin stimulates maternal behaviour in sheep. *Neuroendocrinology*, 46, 56–61.
- Ladd, C. O., Owens, M. J., & Nemeroff, C. B. (1996). Persistent changes in corticotropin-releasing factor neuronal systems induced by maternal deprivation. *Endocrinology*, 137, 1212–1218.
- Liu, D., Caldji, C., Sharma, S., Plotsky, P. M., & Meaney, M. J. (2000). Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinephrine release in the hypothalamic paraventricular nucleus. *Journal of Neuroendocrinology*, 12, 5–12.
- Liu, D., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., et al. (1997). Maternal care, hippocampal glucocorticoid receptor gene expression and hypothalamic-pituitary-adrenal responses to stress. *Science*, 277, 1659–1662.
- Lucas, B. K., Ormandy, C. J., Binart, N., Bridges, R. S., & Kelly, P. A. (1998). Null mutation of the prolactin receptor gene produces a defect in maternal behavior. *Endocrinology*, 139, 4102–4107.
- McBride, W. J., Murphy, J. M., & Ikemoto, S. (1999). Localization of brain reinforcement mechanisms: Intracranial self-administration and intracranial place-conditioning studies. *Behavioural Brain Research*, 101, 129–152.
- Meaney, M. J., Diorio, J., Francis, D., Widdowson, J., LaPlante, P., Caldji, C., et al. (1996). Early environmental regulation of forebrain glucocorticoid receptor gene expression: Implications for adrenocortical responses to stress. *Developmental Neuroscience*, 18, 49–72.
- Pedersen, C. A., Caldwell, J. D., Walker, C., Ayers, G., & Mason, G. A. (1994). Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic area. *Behavioral Neuroscience*, 108, 1163–1171.

- Pedersen, C. A., & Prange, A. J. (1979). Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. *Proceedings of the National Academy of Sciences USA*, 76, 6661–6665.
- Pitkow, L. J., Sharer, C. A., Ren, X., Insel, T. R., Terwilliger, E. F., & Young, L. J. (2001). Facilitation of affiliation and pair-bond formation by vasopressin receptor gene transfer into the ventral forebrain of a monogamous vole. *Journal of Neuroscience*, 21, 7392–7396.
- Plotsky, P., & Meaney, M. J. (1993). Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Molecular Brain Research*, 18, 195–200.
- Russek, L., & Schwartz, G. E. (1997). Feelings of parental caring predict health status in midlife: A 35-year follow-up of the Harvard Mastery of Stress Study. *Journal of Behavioral Medicine*, 20, 1–13.
- Terkel, J., & Rosenblatt, J. S. (1972). Humoral factors underlying maternal behavior at parturition: Cross transfusion between freely moving rats. *Journal of Comparative Physiology and Psychology*, 80, 365–371.
- Wang, Z., Ferris, C. F., & DeVries, G. J. (1994). Role of septal vasopressin innervation in paternal behavior in prairie voles (*Microtus ochrogaster*). *Proceedings of the National Academy of Sciences USA*, 91, 400–404.
- Williams, J., Catania, K., & Carter, C. (1992). Development of partner preferences in female prairie voles (*Microtus ochrogaster*): The role of social and sexual experience. *Hormones and Behavior*, 26, 339–349.
- Williams, J. R., Insel, T. R., Harbaugh, C. R., & Carter, C. S. (1994). Oxytocin administered centrally facilitates formation of a partner preference in prairie voles (*Microtus ochrogaster*). *Journal of Neuroendocrinology*, 6, 247–250.
- Winslow, J., Hastings, N., Carter, C. S., Harbaugh, C., & Insel, T. (1993). A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature*, 365, 545–548.
- Witt, D. M., Winslow, J. T., & Insel, T. R. (1992). Enhanced social interactions in rats following chronic, centrally infused oxytocin. *Pharmacology Biochemistry and Behavior*, 43, 855–861.
- Young, L. J., & Insel, T. R. (2002). Hormones and parental behavior. In J. B. Becker, S. M. Breedlove, and D. Crews, *Behavioral endocrinology* (2nd ed., pp. 331–368). Cambridge, MA: MIT Press.
- Young, L. J., Lim, M. M., Gingrich, B., & Insel, T. R. (2001). Cellular mechanisms of social attachment. *Hormones and Behavior*, 40, 133–138.
- Young, L. J., Nilsen, R., Waymire, K. G., MacGregor, G. R., & Insel, T. R. (1999). Increased affiliative response to vasopressin in mice expressing the vasopressin receptor from a monogamous vole. *Nature*, 400, 766–768.