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EARLY EXPERIENCE AND THE DEVELOPMENTAL PROGRAMMING OF OXYTOCIN AND VASOPRESSIN

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INTRODUCTION

Genetic and epigenetic factors influence social behaviors across the lifespan. Of particular importance are experiences in early life, especially when the nervous system is rapidly developing. At this time neural and endocrine systems may be vulnerable to modifications, with the capacity to re-tune social behavior in later life. The consequences of early experiences may be mediated by endocrine changes originating in the developing organism itself (including systems that rely on steroids or peptides), or through maternal influences, including the epigenetic effects of postnatal parental stimulation (Kaffman & Meaney, 2007). Compounds of maternal origin also might be transmitted through the mother to the infant, either during the prenatal or postnatal period. In addition, in the postnatal period maternal milk contains biologically active compounds, including prolactin (Grosvenor *et al.*, 1993), cortisol (Glynn *et al.*, 2007), and OT (Leake *et al.*, 1981). These hormones, in turn, may regulate the endocrine system of the developing neonate, with long-lasting consequences for behavior and physiology.

Oxytocin is widely manipulated by medical interventions and potentially by different patterns of infant care. It is particularly common to manipulate OT in women for the purpose of regulating the timing of birth. It has been assumed that exogenous OT (pitocin) given to the mother does not pass through the placenta in amounts sufficient to affect the baby. However, the placental barrier may be disrupted during birth. In addition, exposures to pitocin (used to hasten labor), or treatments such as atosiban (an OT antagonist; OTA used to delay labor) vary in timing, duration, and dose. Treatments given to the mother hold the potential to affect the infant indirectly (e.g., by altering the strength or duration of uterine contractions, relative exposure to hypoxia, or the subsequent ability of the mother to lactate or interact normally with her offspring).

The main focus of the studies described here is how early hormonal experience might influence the offspring. Because of the complexity associated with the birth process, the experimental approaches described focus on direct treatments given to offspring during the immediate postnatal period. We also have centered our initial investigations of the effects of early experiences on social behaviors and neuroendocrine processes that are known to be, in later life, peptide dependent or strongly influenced by OT or AVP. We have focused our chapter on studies in prairie voles, a highly social rodent species in which OT and AVP have been shown to be of particular importance to behavior and physiology.

BACKGROUND ON OXYTOCIN AND VASOPRESSIN

OT and AVP are neuropeptides consisting of a six amino acid ring, with a three amino acid tail.

Neurobiology of the Parental Mind

The molecular structures of OT and AVP differ by two of nine amino acids. OT and AVP are synthesized primarily in the central nervous system. Both peptides are found in particularly high concentrations in the paraventricular (PVN) and the supraoptic nuclei (SON) of the hypothalamus. From the PVN and SON, OT and AVP are carried by axonal transport to the posterior pituitary, where they are released into the blood stream. OT and AVP are also released into the central nervous system. Only one OT receptor (OTR) has been described. The same receptor is present in neural tissue and in other parts of the body including the uterus (Gimpl & Fahrenholz, 2001). Three distinct receptor subtypes have been identified for AVP. Of these, the V1a receptor (V1aR), which is found in the brain, has been associated with parental behavior (Bester-Meredith & Marler, 2003; Bales *et al.*, 2007b) and pair bond formation, especially in males (Winslow *et al.*, 1993). Receptors for both OT and AVP are localized in areas of the nervous system that play a role in reproductive, social, and adaptive behaviors, and in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis and the autonomic nervous system. The AVP V1aR exhibits species and individual differences, due at least in part to variations in the promotor region of the gene responsible for expression of that receptor (Hammock & Young, 2005). The OTR also shows species differences (Witt *et al.*, 1991; Insel & Shapiro, 1992) in expression, although the molecular basis of these differences is less well understood.

AVP peptide synthesis within the central nervous system is sexually dimorphic and influenced by developmental exposure to androgens (De Vries & Simerly, 2002). Specifically, cells that synthesize AVP in the amygdala and bed nucleus of the stria terminalis (BNST) and fibers from these cells that extend into the lateral septum, are more abundant in males. In addition, males seem more responsive than females to AVP (Winslow *et al.*, 1993). These findings, as well as research in knock-out mice made defective for the V1aR, support the general conclusion, with regard to behavioral and emotional regulation, that males are more behaviorally dependent than females on central AVP and AVP receptors (Lim *et al.*, 2004; Bielsky *et al.*, 2005).

Recent studies in humans implicate OT and AVP in the response to stressors, repetitive behaviors and anxiety (reviewed, Carter, 2007). OT may reduce anxiety and reactivity to stressors, as well as repetitive behaviors. Research in animals also implicates OT in maternal behaviors, sexual behavior, social recognition, and social contact, and OT can facilitate pair bonding. AVP, working in conjunction with OT, may be of particular relevance to male social behaviors including parental behavior, pair bond formation, and mate guarding. The effects of AVP probably differ as a function of dose, with lower doses being anxiolytic and higher doses anxiogenic (Carter, 2003).

Sex differences in the AVP system could have broad consequences for behavior and physiology, forming an underlying substrate for processes that are typically attributed to the effects of androgens. It is possible that in males both peptide synthesis and receptor functions are primed by early exposure to androgens, including exposure in the early postpartum period. The capacity of OT manipulations in early life to have long-term effects on neural systems involving AVP would be expected to have sexually dimorphic consequences for many aspects of physiology and behavior. However, in comparison to OT, the possible behavioral functions of AVP in females have received less attention, so these conclusions must remain tentative.

SIMILARITIES OF FUNCTION BETWEEN OT AND AVP

OT and AVP have some overlapping functions, possibly because they can bind to each other's receptors (Barberis & Tribollet, 1996). In some situations the behavioral actions of OT and AVP appear similar, but more often the behavioral effects of OT and AVP seem to be opposing (Carter, 1998). In general OT is associated with increases in sociality, reductions in mobilization, and can induce reductions in the activity of the HPA axis, while AVP may also facilitate certain forms of social behavior, but is often associated with increased arousal and elevations in the activity of the HPA axis. Female reproductive strategies including birth, lactation, and immobile sexual posters may be facilitated by OT, while active coping and overt defensive behaviors may rely on AVP (Porges, 1998). The differential actions of OT and AVP may be of particular importance to understanding sex differences in social behavior.

During critical periods in development, manipulations of OT or AVP may influence the

expression of these same peptides, as well as their receptors, with life-long behavioral consequences. However, it is not a simple matter to untangle differential functions of OT and AVP, especially using available pharmacological tools. In addition to the ability of OT and AVP to bind to each other's receptors (Barberis & Tribollet, 1996), the OTA widely used in behavioral studies is not totally selective for the OTR and also affects the AVP V1aR.

EFFECTS OF EARLY HANDLING IN PRAIRIE VOLES

In prairie voles we find that small manipulations, created by different methods of handling the parents or offspring in the postnatal period, can have life-long behavioral consequences (Bales *et al.*, 2007a; see Table 27.1). In a series of experiments, we have examined the effects of differential early behavioral treatments of the infants and family during the neonatal period on later behavioral and neuroendocrine responses in prairie voles. Compared to studies done in rats, which typically involve periods of separation from the mother, the handling manipulations described here in voles were very subtle. During a regular cage change, usually within the first day of life, the parents and offspring were transferred to a fresh cage using a cup, either without direct "handling" of the animals (MAN0) or by picking them up by the scruff of the neck for between cage transfers (MAN1). Because pups of this species have milk teeth, they are typically attached to their mother and are transferred with her when she is moved. Observations during the postnatal periods suggest that *mild* disruptions of the family, such as those experienced in the MAN1 treatment, are followed by increases in parental behavior, especially on the part of the mother, but also by the father. In contrast, the MAN0 treatment was associated with lower levels of parental stimulation of the young. Furthermore, excess stimulation in early life, induced by repeated neonatal handling (picked up three times on the first day of life; MAN1 × 3) seemed to distract the parents from showing pup-directed behaviors. Thus, repeated handling, especially in the first days of life, produced immediate and long-term effects that were in some respects similar to those observed in the MAN0 condition (Boone *et al.*, 2006; Tyler, Carter, and Bales, in preparation). {AQ1}

Behavioral testing as a function of early experienced, summarized below, was typically conducted in the postweaning, juvenile period (approximately 21–23 days of age) or in adulthood (at approximately 60–90 days of age). The postweaning period is especially important in prairie voles since the willingness of animals to show social responses within the family, including alloparental behavior (care for

TABLE 27.1 A summary of effects of early experience on prairie voles. Changes are in comparison to MAN1 animals, whose parents are picked up by the scruff of the neck during cage changes. Parents of MAN0 animals are handled with a cup during early cage changes, while parents of MAN1 × 3 animals are picked up by the scruff three times on PND1

	MAN0	MAN1 × 3
Alloparental care	Low in males, normal in females	Low in MAN1 × 3 males
Anxiety (reduced EPM exploration)	High in both sexes	High in males
Pair bonding	Inhibited in females, but not in males	Not measured
OT-ir, PVN	No change from MAN1	Males lower than MAN1
OT-ir, SON	Lower than MAN1	Males lower than MAN1
Baseline corticosterone	No change from MAN1	No change from MAN1
Stress changes in corticosterone	Longer to return to baseline	Higher in MAN1 × 3 males than MAN1 males
Parental care toward own offspring	Lower licking of pups for both males and females	Not measured

offspring that are not their own) can have impact for the development of extended families. In nature increased availability of caretakers is associated with high levels of infant survival (Getz & Carter, 1996).

EARLY EXPERIENCE ALSO ALTERS SUBSEQUENT BEHAVIOR AND MEASURES OF OT AND THE OTRS

As a function of early differential handling, male and female voles both show subsequent changes in behavior; however, the nature of these effects is often sexually dimorphic. For example, animals that received minimal manipulations during the first week of life, here described as "unmanipulated" or MAN0, have been compared to animals exposed to mild stimulation (MAN1). MAN0 males were later less likely to exhibit alloparental care toward pups (see Figure 27.1) and in later life were less exploratory in the open arm of an elevated plus maze (EPM), often used to index anxiety (Bales *et al.*, 2007a). In contrast, MAN0 vs. MAN1 female voles did not differ in alloparental behavior (measured in the postweaning period). However, in adulthood MAN0 females were less likely than MAN1 females to form pair bonds, but were also more anxious, as measured by behavior in the EPM. The first week appears to be a critical period for effects of differential handling, and animals that were first manipulated on postnatal day 8 were similar to MAN0 animals (Bales *et al.*, 2007c).

Among the long-lasting effects of early handling were relative differences in the expression of the OT peptide in the central nervous system, which might account for at least some of the reduced sociality in MAN0 animals that received reduced handling disturbance in early life. In comparison to MAN1 animals, MAN0 voles of both sexes had significantly fewer OT immunoreactive cells in the supraoptic nucleus at 21 days of age (Bales *et al.*, 2007c). Pair bonding in female prairie voles is facilitated by OT and blocked in adulthood by OTA (Williams *et al.*, 1994). Thus, experiences that reduce available OT could produce animals that are less likely to pair bond.

INTERGENERATIONAL EFFECTS OF EARLY EXPERIENCE

There is increasing evidence from rats that early experience, possibly mediated by differential parenting, can be transmitted to subsequent generations of offspring (Francis *et al.*, 1999; Pedersen & Boccia, 2002). Using the handling model described above, infants reared under MAN0 and MAN1 conditions were as adults mated in various combinations (MAN0 female × MAN1 male, MAN0 male × MAN1

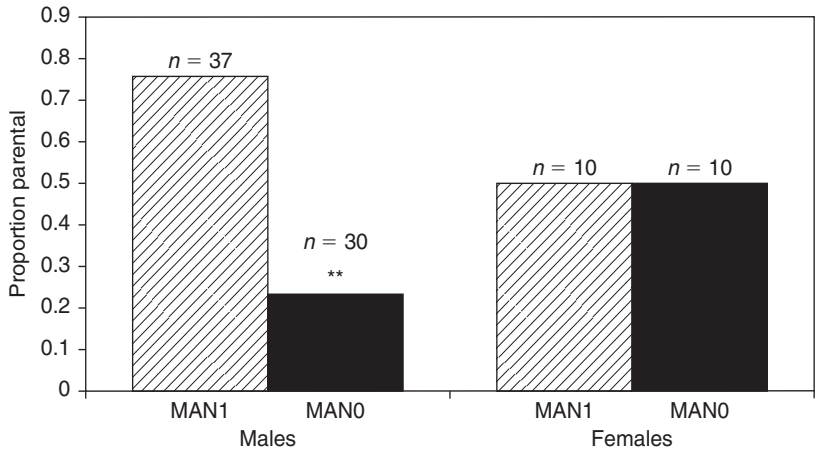

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FIGURE 27.1 Proportion of animals that behaved parentally as a function of neonatal manipulation group ($n = 67$, $\chi^2 = 29.16$, $p < 0.0001$). Data for males include result of three replications in two different laboratories. Data for females are from a single experiment carried out at the University of Illinois at Chicago (*Source*: Reproduced from Bales *et al.* (2007a)).

female, MAN1 male × MAN1 female) and examined for possible differences in sexual behavior, reproductive success, and parental behavior toward their own offspring. While most effects on reproduction fell short of statistical significance, pairs containing a MAN0 male took longer to produce their first litter, although the frequencies of sexual activities, such as bouts of mounting and intromission, were actually higher in pairs containing MAN0 than MAN1 males. Females in pairs with either MAN0 males or MAN0 females licked their pups less than MAN1 × MAN1 pairs (Bales *et al.*, 2007c). Earlier studies in prairie voles have suggested that OT can inhibit male sexual behavior in this species (Mahalati *et al.*, 1991), while OT may enhance parental behavior in both sexes in this species (Bales *et al.*, 2004b, 2007d). In the second generation all animals were treated like the MAN1 condition. The offspring of the second generation, reared by parents with either a MAN0 father or mother, displayed significantly lower frequencies of alloparental behavior than that of pairs reared by MAN1 × MAN1 parents (Bales *et al.*, 2007c). Thus, this demonstrates that these effects of early handling in prairie voles can be passed to the next generation.

THE CONSEQUENCES OF EARLY EXPERIENCE FOR PEPTIDE RECEPTORS

There is increasing evidence that the long-lasting effects of social experiences may be in part mediated via developmental changes in receptors for OT or AVP. In rats, experience-based changes in both the OT and V1aRs are sexually dimorphic (Champagne *et al.*, 2001; Francis *et al.*, 2002), with more marked changes in OTRs in females and the V1aRs in males. In mice, the consequences of genetically induced deficiencies in the V1aR are also gender specific, having greater consequences in males than females (Lim *et al.*, 2004; Bielsky *et al.*, 2005). In addition, in rats prenatal stress is associated in later life with reductions in the OTR (Champagne & Meaney, 2006).

Our preliminary data indicate that the manipulations of early experience described above also have long-term effects on peptide receptors, particularly OTRs. These effects are not always in the predicted directions and also appear to be sexually dimorphic (Bales *et al.*, 2007c).

DEVELOPMENTAL MANIPULATIONS OF OXYTOCIN

The OT system has exceptional plasticity and may be affected by a variety of developmental factors. OT neurons in adult rats have been described as "immature." These neurons have the capacity to change shape and form new synapses, in part through changes in the glia that normally separate neurons. Both OT and AVP may directly or indirectly influence cellular growth, death or motility, inflammation or differentiation. The potential to remodel the nervous system, especially in early life, offers another process through which OT or AVP may have effects on physiology and behavior. The OTR is also susceptible to epigenetic regulation, for example, by silencing genes via methylation (Kimura *et al.*, 2003; Szyf *et al.*, 2005). The capacity of genes that code for receptors to be silenced or otherwise modified in early life may be particularly relevant to understanding the long-lasting consequences of early experiences, whether originating as behavioral or hormonal experiences.

Possible short-term and long-term consequences for young mammals for exposure to exogenous OT or OTAs have been investigated, primarily in prairie voles (Carter, 2003, 2007). The data collected thus far in animal models have confirmed our original hypothesis that exposure to either OT or an OTA during development can have both immediate and long-lasting consequences for behavior and physiology.

In the experiments described below neonatal prairie voles were treated with either OT (usually 1 mg/kg) or OTA (0.1 mg/kg). Control treatments included either a sterile saline injection or handling. Treatments were given as an intraperitoneal injection on postnatal day 1 (PND1); infants were marked by toe-clipping and then returned to their parents. Litters comprised animals of both sexes receiving one experimental treatment (OT or OTA) and one control treatment (saline or handling).

IMMEDIATE OR SHORT-TERM EFFECTS OF NEONATAL OT OR OTA

Neural Activation Following Neonatal Treatment

When neonatal voles were exposed on PND1 to OT or OTA, changes were detected in neural

activation, as measured by changes in c-Fos expression within 1 h or less of treatment (Cushing *et al.*, 2003b). Both OTA and OT treatment caused changes in c-Fos expression in the supraoptic area (SON), with OT increasing c-Fos in males while OTA increased c-Fos in females. Additionally OTA decreased c-Fos expression in the medial dorsal thalamic nucleus in females, but not males (Cushing *et al.*, 2003b).

Behavioral and Endocrine Changes in Neonates

In female voles treatment on PND1 with OT or OTA (vs. control procedures) produced changes in corticosterone, and in reactivity to acute social isolation in animals tested on PND8 (Kramer *et al.*, 2003). Females receiving OTA (PND1), relative to controls, had on PND8 elevated basal plasma corticosterone and also vocalized significantly less when isolated from their family. The effect of OTA differed as a function of single vs. repeated injection procedures; females given repeated exposure to OTA (daily injections PND1–7) vocalized significantly more during social isolation than did controls injected daily with saline or handled on PND1–7.

LONG-TERM EFFECTS OF NEONATAL OT AND OTA

Alloparental Behavior, Sociality, and Reproduction in Males

The long-term consequences of transient neonatal exposure to comparatively low doses of OT (1 mg/kg on PND1) tended to be similar to those seen when OT was administered during adulthood. When effects were detected, neonatal OT was associated with increased levels of sociality. For example, males receiving a single neonatal exposure to OT (PND1) formed pair bonds more quickly than animals receiving either no treatment or a saline injection (Bales & Carter, 2003b). The behavioral effects of neonatal OTA (0.1 mg/kg on PND1) often (but not always) were in directions that were opposite to those of OT; in general, reductions in social behavior in later life were seen in animals neonatally exposed to OTA. Males receiving OTA (PND1) were in later life less alloparental and more aggressive toward pups (Bales *et al.*, 2004c). OTA-treated males also tended to be less aggressive toward same-sexed strangers, which in this species may be indicative of increased anxiety (Bales *et al.*, 2004c).

When a higher neonatal dosage of OT (4 mg/kg) was administered, males also formed a pair bond (Figure 27.2). However, males receiving an intermediate dosage of 2 mg/kg did not demonstrate a preference for either the partner or the stranger, suggesting that the effects of OT exposure are not necessarily linear.

We also carried out a preliminary study in males in which we attempted to remediate the deficits in alloparental behavior produced by PND1 exposure to OTA (0.1 mg/kg) (or saline), and using a subsequent treatment on PND8 with OT (1 mg/kg) (or saline) (Bales & Carter, unpublished data). Each male received two injections, including OT, OTA, or SAL (saline, the vehicle control) in the following combinations SAL/SAL, OTA/SAL, or OTA/OT. Alloparental care, measured by huddling over pups on PND21, was low in animals that received OTA/SAL (11%), but was improved to 45% by PND8 exposure to OT (i.e., OTA/OT).

In another study of male prairie voles PND1 exposure to either OTA or OT also was capable of disrupting subsequent sexual behavior and reproductive potential (Bales *et al.*, 2004a). Both OT- and OTA-treated animals, showed in adulthood, indications of deficiencies in sperm transport; OT-treated males also demonstrated temporal differences in mating behavior (Bales *et al.*, 2004a).

Social Behavior in Females

In general females seemed to be less sensitive than males to the effects of PND1 treatments with OTA (0.1 mg/kg) or OT (1 mg/kg). However, females were affected by higher dosages of exogenous OT when given on PND1. OT (2–8 mg/kg) tended to slightly enhance certain aspects of sociality, including postweaning alloparental behavior. As adults these females were also tested under conditions that usually reveal selective social behaviors, including the partner preference behaviors used to index pair bond formation in voles (Williams *et al.*, 1992). In the case of partner preferences, the effects of neonatal exposure to OT were dose dependent and bimodal. Partner preferences were significantly enhanced in the group that

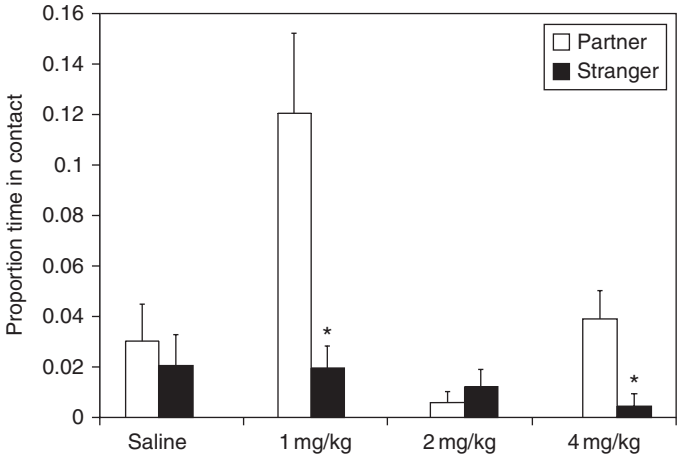

FIGURE 27.2 Effects of early OT treatment in *males* on preference for a partner vs. a stranger measured as a proportion of test time spent in side-to-side contact. Data presented here are from two different studies. 1 mg/kg data are from Bales and Carter (2003b). 2 mg/kg and 4 mg/kg data are previously unpublished, while saline data are combined for the two studies. Both 1 mg/kg ($t(12) = 2.23, p = 0.05$) and 4 mg/kg ($t(10) = 4.29, p = 0.002$) groups showed a statistically significant within-group partner preference.

received neonatal OT at 2 mg/kg, but were disrupted by higher doses of OT (4 mg/kg), and at a very high dose (8 mg/kg) OT-treated females later showed a preference for a stranger rather than the familiar partner. Once more these data reveal the capacity of early peptide exposure to alter later social interactions, but in the case of exposure to the highest dose the familiar animal later was either less preferred or possibly even aversive (Bales *et al.*, 2007d; see Figure 27.3). Alloparenting also varied with neonatal OT dosage, although the optimal dosage for facilitation of alloparenting differed from that for partner preference, suggesting the possibility that neonatal peptides act on these two forms of social behavior through mechanisms that are not identical.

Neonatal OTA in Females

The effects of neonatal OTA (PND1 at 0.1 mg/kg) were generally less disruptive in females than males. We have hypothesized that this comparative insensitivity of females or sensitivity of males to neonatal OTA may be due to OTA-induced disruptions in AVP-dependent processes, which are more critical to male behavior than female behavior. However, in females one interesting effect of neonatal OTA was detected: when female prairie voles were exposed to OTA (0.1 mg/kg on PND1) and then as adults paired with a male, neural activation of the central amygdala was increased (as measured by increased c-Fos expression). In prairie voles the central amygdala is not normally activated after pairing with an individual of the opposite sex, and activation of this area may reflect an increased state of emotional arousal or even fear in the OTA-treated females. No treatment effects were apparent in males in that study (Kramer *et al.*, 2006). The mechanisms for this apparent sex difference remain to be studied, but might in this case be due in females to OTA-induced changes in systems that rely on OT.

EFFECTS ON NEONATAL OT OR OTA ON BRAIN HORMONES AND RECEPTORS

Effects on Brain Peptides

In female prairie voles a single PND1 exposure to either OT (1 mg/kg) or OTA (0.1 mg/kg) was associated with increased central OT, measured at the time of weaning (PND21). In males there were no significant effects of either neonatal OT or OTA on central OT, but changes were detected in the AVP system. In males following neonatal OTA there was an apparent decrease in AVP, also measured on PND21 (Yamamoto *et al.*, 2004). In a related study, effects of early OT (1 mg/kg and lower) on OT

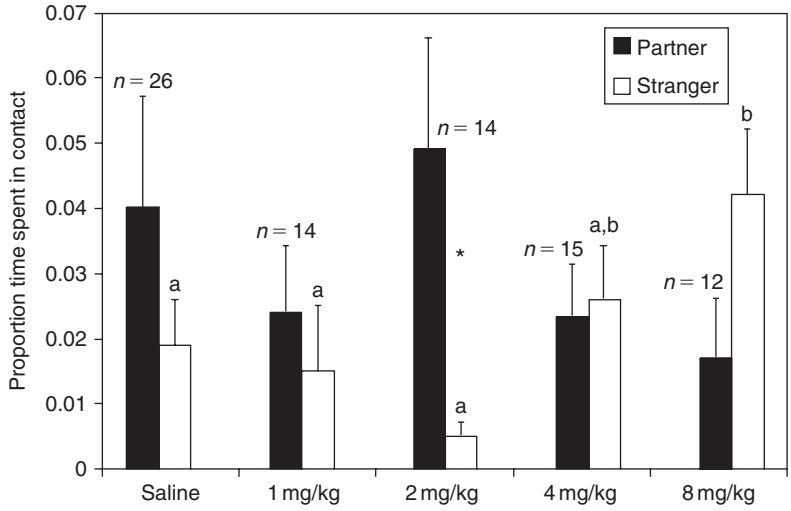

FIGURE 27.3 Effects of early OT treatment in *females* on preference for a partner vs. a stranger, measured as a proportion of test time spent in side-to-side contact. Proportion of test time spent in side-to-side contact with a stranger differs significantly by treatment ($\chi^2(4) = 10.76$, $p = 0.029$), as does the difference between time spent with the partner and time spent with the stranger ($\chi^2(4) = 10.05$, $p = 0.04$) (*Source*: Reproduced from Bales *et al.* (2007d)). {AQ5}

production in the PVN were no longer detectable in adulthood (Kramer *et al.*, 2007).

AVP is important to a variety of male behaviors in this species, including pair bond formation and mate guarding (Winslow *et al.*, 1993), male parental behavior (Wang *et al.*, 1994), and the management of anxiety or reactions to stressful experiences (Carter & Keverne, 2002). Thus, sexually dimorphic consequences of differential availability of either AVP or OT may be explained in part by the capacity of these peptides to be altered by differential peptidergic experiences in early life.

Effects on Peptide Receptors

Brain tissues from animals exposed to these same doses of neonatal OT or OTA were studied using autoradiography for possible changes in the OTR, AVP receptor (V1aR), as well as dopamine receptors (D1 or D2) (Bales *et al.*, 2007b). Effects on the OTR and dopamine receptors of neonatal OT or OTA manipulations were comparatively small, and not statistically significant. However, manipulations of OT on PND1 were associated with a pattern of regional changes in the V1aR system. Sex differences in the distribution of V1aRs in untreated prairie voles were not observed, consistent with earlier reports in this species. However, neonatal manipulations of OT produced long-lasting, sexually dimorphic effects on V1aR levels (Figures 27.4 and 27.5). Particularly striking were changes in the ventral pallidum in OT-treated males – increases in V1aR binding. In contrast, OT-treated females showed decreases in V1aR binding in this region. The ventral pallidum has been implicated in pair bond formation in male prairie voles, and is a region in which AVP V1aRs and dopamine-based "reward" processes may coexist (Young *et al.*, 2005b). Long-lasting increases in the V1aR in the ventral pallidum could help to explain the enhanced sociality of males receiving neonatal OT. Reduced AVP binding following neonatal OTA, especially in males that depend on AVP, might help to explain reductions in later social behavior in OTA exposed males (Carter, 2007).

Males exposed neonatally to OTA also showed reductions in V1aR binding in several other brain regions that have been implicated in both social behavior and emotionality, including the BNST, medial preoptic area, and lateral septum. AVP acting in these same brain regions has been associated later in life with male parental care in several species (Wang *et al.*, 1994; Bester-Meredith & Marler, 2003) and with male–male aggression (Marler *et al.*, 2003), which in pair-bonded prairie voles is

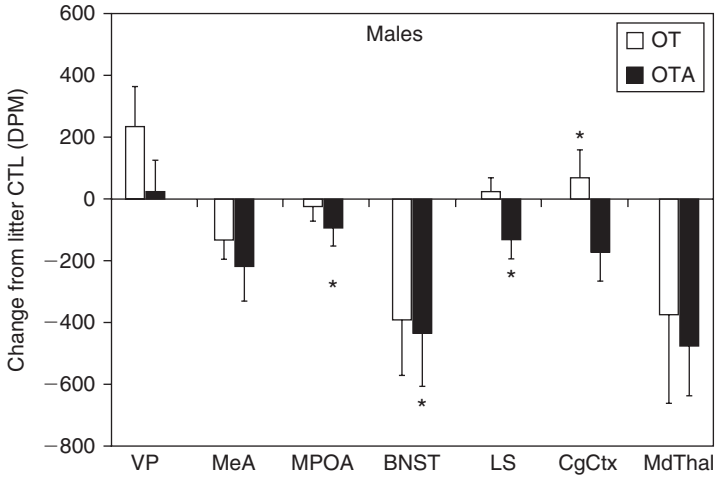

FIGURE 27.4 Effects of early OT or OTA treatment in *males* on V1aR binding. *$p < 0.05$. VP, ventral pallidum; MeA, medial amygdala; MPOA, medial preoptic area; BNST, bed nucleus of the stria terminalis; LS, lateral septum; CgCtx, posterior cingulate cortex; MdThal, mediodorsal thalamus (*Source*: Reproduced from Bales *et al.* (2007b)).

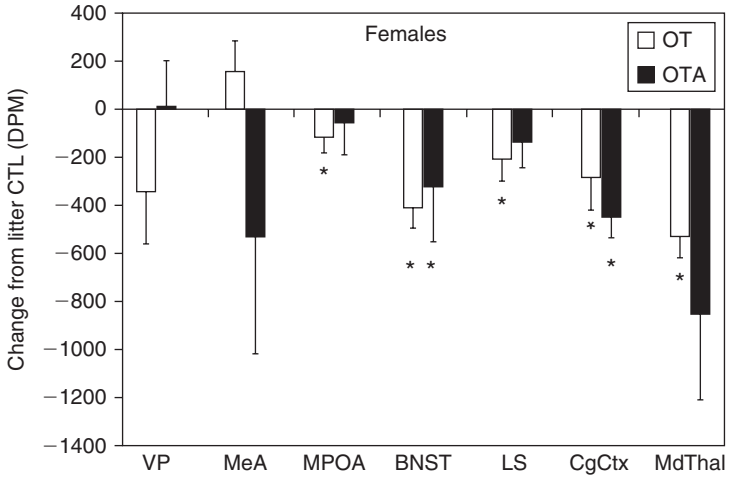

FIGURE 27.5 Effects of early OT or OTA treatment in *females* on V1aR binding. *$p < 0.05$. VP, ventral pallidum; MeA, medial amygdala; MPOA, medial preoptic area; BNST, bed nucleus of the stria terminalis; LS, lateral septum; CgCtx, posterior cingulate cortex; MdThal, mediodorsal thalamus (*Source*: Reproduced from Bales *et al.* (2007b)).

a component of mate guarding (Winslow *et al.*, 1993). We have observed that male, but not female, prairie voles treated neonatally with OTA showed reduced alloparental behavior (Bales *et al.*, 2004c), and tended to show lower levels of same-sex aggression than control males (Bales & Carter, 2003a), supporting a role for AVP and/or the V1aR in these behaviors.

Neonatal Manipulations of AVP also Affect Subsequent Social Behaviors

Earlier studies in prairie voles have revealed that neonatal exposure to AVP (in this case given as daily injections in the first week of life) were associated with a later dose-dependent increase in same sex aggression, especially in males (Stribley & Carter, 1999). In contrast, neonatal

exposure to an AVP antagonist was associated in later life with very low levels of aggression. Other aspects of behavior including exploration in an EPM and partner preference formation were not significantly affected by either neonatal AVP or the AVP antagonist. The effects of neonatal AVP manipulations on V1aR binding have not yet been examined in voles. However, because these studies involved repeated daily injections and handling, the effects of AVP cannot be compared directly to those of OT described here, in which treatments were typically given once on the first day of life.

Early Exposure to Gonadal Steroids Facilitates the Response of Adult Male Prairie Voles to Exogenous AVP

In adult male prairie voles AVP plays a major role in pair bond formation (Winslow *et al.*, 1993). However, following neonatal castration, males tested as adults did not form partner preferences in response to centrally administered AVP. Neonatal treatment with testosterone restored the ability of castrated male prairie voles to respond to AVP in adulthood, in this case with the formation of a partner preference. Replacement of testosterone in adulthood did not restore partner preference formation in response to AVP in neonatally castrated males, suggesting once again that the postnatal period is a time when animals are especially sensitive to hormonal manipulations. Interestingly, neonatal castration did not affect the distribution of AVP V1aR, as measured by autoradiography (Cushing *et al.*, 2003a). This conclusion is also supported by the general absence of sex differences in either the OTR or AVP V1aR that have been reported in several studies. The lack of an effect of neonatal castration on the V1aR is in contrast to the significant and sexually dimorphic effects of neonatal manipulations of OT on the V1aR (Bales *et al.*, 2007b). These findings suggest the hypothesis that changes in peptides in early life may be more relevant than changes in gonadal steroids in regulating later peptide receptor distribution.

Estrogen Receptors also Affected by Neonatal OT or OTA

Among the other long-lasting changes that followed neonatal manipulations of OT or OTA were effects on the later expression of estrogen receptors (ERα) (Cushing & Kramer, 2005; Kramer *et al.*, 2007). In female prairie voles, neonatal OT increased the later expression of ERα in the ventromedial hypothalamus, while OTA decreased ERα in the medial preoptic area (Yamamoto *et al.*, 2006). A follow-up study indicated that during the neonatal period OT may affect the expression of ERα by influencing the production of ERα mRNA (Pournajafi-Nazarloo *et al.*, 2007a). On the day of birth female prairie vole pups were treated with OT, OTA, or saline (as above). Within 2 h of treatment, OT significantly increased ERα mRNA expression in the hypothalamus and hippocampus, but not the cortex (measured by RT-PCR). In contrast, neonatal exposure to OTA was associated with a later reduction in the expression of ERα mRNA in the hippocampus. Neonatal treatment did not affect the later expression of the ERα mRNA. Regional specific changes in ERα mRNA expression in females are consistent with studies examining the behavioral and physiological effects of neonatal manipulation of OT in females. Significant effects on ERα of neonatal OT manipulations were not detected initially in males (Cushing & Kramer, 2005). However, in a subsequent study exposure to neonatal OTA was associated with an increase in ERα in the BNST (Kramer *et al.*, 2007). These studies again support the hypothesis that manipulations of OT can have organizational effects, and that the effects of OT are sexually dimorphic. However, in the case of ERα females tended to be more sensitive than males, at least to the effects of neonatal OT. ERαs play a role in a variety of social and reproductive behaviors; alterations in ERαs would be expected to have broad consequences for many systems upon which steroid hormones act both during development and also in adulthood, and are possible mediators of at least some of the effects of neonatal manipulations of peptides.

EFFECTS OF NEONATAL OT OR/AND OTA IN RATS

Reproductive and Endocrine Effects

An extensive analysis of the consequences of neonatal peptides in species other than prairie voles remains to be conducted. However, in rats

comparable neonatal manipulations of OT did have long-lasting, sexually dimorphic effects on pituitary levels of OT (Young *et al.*, 2005a). In female rats, treatment with OTA on PND1 significantly decreased pituitary OT levels as adults. In contrast, OTA treatment (PND1) in males resulted in increased pituitary OT levels in adulthood, at least when compared to males treated with OT (PND1). In rats receiving daily OT in the first week of life (PND1–7), subsequent puberty was delayed. This treatment with OT significantly delayed the age at vaginal opening and the age at first estrus (Withuhn *et al.*, 2003).

Cardiovascular and Autonomic Effects of Neonatal OT

In rats early exposure to OT (1 mg/kg) (daily on PND1–14) was followed in later life by increased abundance of $\alpha 2$ adrenergic receptors (Diaz-Cabiale *et al.*, 2004). These changes were most obvious in the hypothalamus and the amygdala, as evaluated by quantitative receptor autoradiography. In offspring from *ad libitum* fed dams, OT treatment significantly increased the density of $\alpha 2$-agonist binding sites in the nucleus tractus solitarius and in the hypothalamus. In addition, in offspring from food-restricted dams, OT treatment also produced a significant increase in indicators of $\alpha 2$-activity, supporting the more general hypothesis that chronic exposure to OT in early life might be capable of reducing sympathomimetic activity and enhancing parasympathetic functions, with broad significance for behavioral and emotional reactivity in later life.

Neonatal exposure to OT in rats also had consequences for gene expression in heart tissue (Pournajafi-Nazarloo *et al.*, 2007b). In this study female and male rats received either OT or saline on PND1. Hearts were collected either on PND1, 1 h following injection, or PND21. At these times the expression of mRNAs (using RT-PCR) was measured for the OTR, atrial natriuretic peptide (ANP), inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS), ERα, and ERβ. OT treatment significantly increased gene expression in the heart for OTR, ANP, and eNOS measured on PND1 in both males and females. As a function of OT treatment, ERα increased only in females. Significant treatment effects were no longer detected in PND21 animals.

SEX DIFFERENCES

The consequences of developmental manipulations of OT are often sexually dimorphic, relying on different neural substrates in males and females (Carter, 2007). One possible reason for the differences between the sexes may be because in males the developmental effects of OT or OTA are mediated, at least in part, through differential effects on AVP (Yamamoto *et al.*, 2004) and/or its receptor (Bales *et al.*, 2007b). In both sexes OT manipulations, either OT (1 mg/kg) or OTA (0.1 mg/kg), did not have a significant effect on the OTR or dopamine receptor (D1 or D2) as measured by autoradiography (Bales *et al.*, 2007b).

Because parental care behavior was a major dependent variable in these studies, we also have analyzed the impact of factors, including peptide administration and stressful experiences, on adult parental behavior. Adult male parental behavior was only eliminated by high dosages of both OTA and a vasopressin V1aR antagonist (Bales *et al.*, 2004; see Figure 27.6). In one study in adults, prairie voles were either stressed (by a 3-min swim) or not stressed, and then tested with infants. Components of adult male alloparental responses toward infants were increased by the prior swim, while female alloparental behaviors were unaffected (Bales *et al.*, 2006; see Figures 27.7 and 27.8). It is likely that AVP, as well as OT, were released during these stressors. Sex differences in central AVP and sex differences in the physiology of parental behavior (Wang *et al.*, 1998; Bales *et al.*, 2004b), and specifically the reliance of males on AVP, may help to explain the capacity of stressful experiences to enhance male alloparental behavior. {AQ2}

DEVELOPMENTAL SIGNALING CONSEQUENCES OF NEUROPEPTIDES

Maternal OT can act as a signaling mechanism between the mother and fetus. Based on studies in rats, maternal OT, released during birth, also triggers a transient switch in GABA signaling in the fetal brain from excitatory to inhibitory. *In vivo* administration of an OTA before delivery prevented this switch of GABA activity in fetal neurons, and aggravated the severity of anoxic episodes. Thus, it appears that maternal OT inhibits fetal neurons and increases their

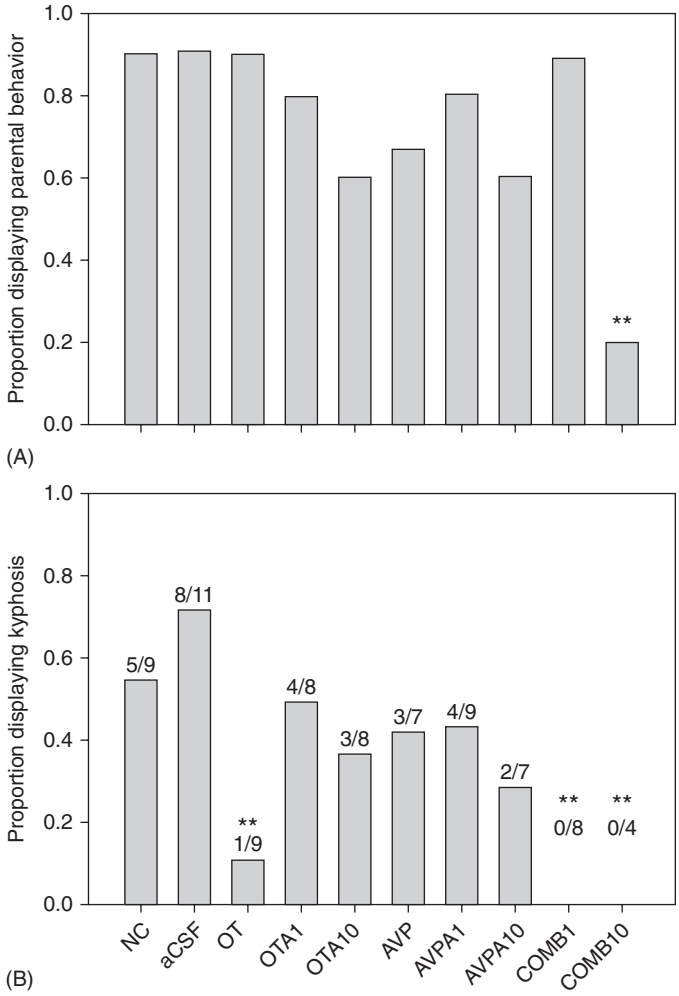

FIGURE 27.6 (A) Proportion of male voles responding parentally (displaying kyphosis, non-kyphotic contact, retrieving, or licking or grooming) toward infants. Significance was assessed in comparison to the aCSF group. Treatments are as follows: control (NC – no cannulation), aCSF (artificial cerebrospinal fluid), OT (oxytocin), OTA1 (1 ng oxytocin antagonist), OTA10 (10 ng OTA), AVP (arginine vasopressin), AVPA1 (1 ng AVP antagonist), AVPA10 (10 ng AVPA), COMB1 (combination treatment, 1 ng AVPA and 1 ng OTA), and COMB10 (combination treatment, 10 ng AVPA and 10 ng OTA). Only the COMB10 group differed significantly from the aCSF control (Fisher's exact probability test, $^{**}p = 0.002$). All group sizes are equal to 9–11 males. (B) Proportion of male voles (out of those not attacking) that displayed kyphosis. Significance was assessed in comparison to the aCSF group. **Different from aCSF group at $p < 0.05$ (*Source*: Reproduced from Bales *et al.* (2004b)).

resistance to hypoxic insult (Tyzio *et al.*, 2006). In addition, the birth-related surge in OT also helps to regulate the synchronization of the fetal hippocampal neurons, possibly allowing the transition from prenatal to postnatal life (Crepel *et al.*, 2007). Such changes would be expected to have consequences for both emotional and cognitive functions.

EARLY EXPERIENCE IN THE CONTEXT OF NATURAL HISTORY

Laboratory experiments have revealed that relatively subtle changes in early experience have long-term and sexually dimorphic consequences for the later social behavior of the offspring.

{AQ5} **FIGURE 27.7** Proportion of animals displaying parental behavior after a swim stressor or no swim stressor ($n = 10$ animals/group). Males were more likely to display parental behavior following stress (logistic regression, likelihood ratio $\chi^2 = 3.4522$, $p = 0.032$; one-tailed), while females were not (*Source*: Reproduced from Bales *et al.* (2006)).

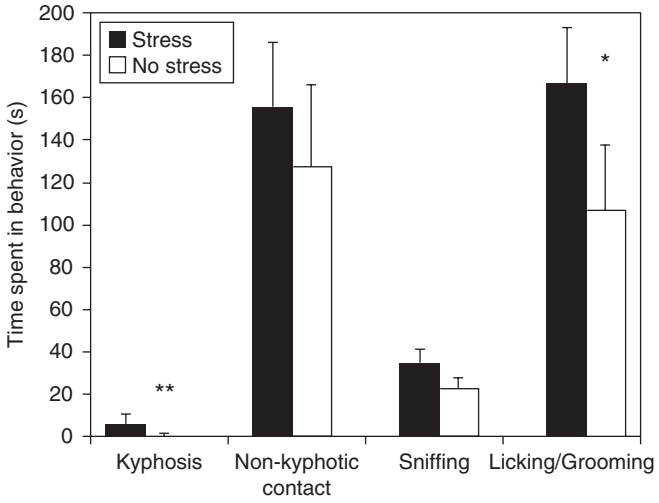

FIGURE 27.8 Time spent (seconds) by males in parental care behaviors, following exposure or no exposure to stress $^{**}p < 0.05$, $^{*}p < 0.1$ (*Source*: Reproduced from Bales *et al.* (2006)).

For example, disruption of the family associated with a single handling in the immediate postnatal period (MAN1) was associated in later life with increased alloparental behavior in male offspring and an increased tendency to form new pair bonds in female offspring. Conversely, reduced handling produced offspring that appeared anxious. Under these conditions female offspring were less likely to form pair bonds. Additional handling or manipulation in the neonatal period did NOT produce further increases in alloparenting, and in fact appeared to inhibit certain aspects of later social behavior. Preliminary data suggest that repeatedly manipulated animals (both parents and offspring) showed increased fear and anxiety. For example, when families were disturbed three times on PND1 (MAN1 $\times$ 3), males in particular were later less alloparental compared to MAN1 males. Under some conditions, the more frequently disturbed animals, especially males, showed reduced alloparenting or even attacked pups. Upon an initial disruption most mothers increased their interactions with their

pups. However, following repeated disruptions mothers appeared agitated and less attentive to pups (Boone *et al.*, 2006).

These findings may be best interpreted in the context of circumstances that occur under field conditions. In nature prairie voles may live under a variety of social circumstances with consequences for the care of young. A percentage of males and females form pair bonds, live together, and produce their own offspring. However, it has been estimated that approximately 70% of young animals do not form new families, but remain instead as part of a communal breeding group (Getz & Carter, 1996). Under conditions of high mortality or mate abandonment, females may become single parents. However, even if the father is not present, older offspring can help to care for their younger siblings. Variation in the composition of the family would result in naturally occurring differences in the amount of stimulation received by offspring. Such variation could be translated into hormonal messages and epigenetic modifications that would determine whether a young animal would remain in the natal nest, where it would be an alloparent, or alternatively attempt to find a partner and establish a new pair bond and family. Variation in AVP V1aRs has been observed in field caught animals, usually exceeding the variation in animals reared under routine laboratory conditions (Phelps & Young, 2003), which may approximate the MAN1 conditions of our experimental manipulations of early experience.

TRANSLATIONAL IMPLICATIONS OF PERINATAL MANIPULATIONS OF OT

Of particular concern and largely unstudied in humans are the possible consequences of exposure to exogenous peptides, including OT, in the perinatal period. The use of synthetic OT (Pitocin) has grown in prevalence as a method for inducing or augmenting labor. As just one example, Pitocin was used in 93% of the approximately 10,000 births at Northwestern Hospital in Chicago in 2005 (Cynthia Wang, personal communication). Prematurity also continues to be a major medical problem and treatments attempting to delay labor hold the potential to affect the fetus. OTAs, such as Atosiban (currently not approved in the United States, but available in 43 other countries), have been used to delay or prevent premature labor (Husslein, 2002). Other OTAs are being developed as methods for delaying parturition. However, the consequences for human brain and behavior of early OT manipulations, including exogenous OT, or of the use of OTAs remain largely unknown.

The possible effects of early experience or hormonal manipulations also have not been systematically studied in human development. The neural systems that are altered by neonatal peptide manipulations in prairie voles are evolutionarily ancient and have broad behavioral and physiological actions. Our studies suggest that these systems may be accessible to change and thus vulnerable during development to neural changes that could have long-lasting consequences. Results from the present study suggest the need for a deeper understanding of the mechanisms through which manipulations in endogenous or exogenous peptides might affect neuroanatomy, physiology, and behavior. For example, manipulations in OT could have long-lasting consequences for behavioral endophenotypes, including sociality and reactivity to stressors, that are core to personality types and in extreme cases to several psychiatric disorders, including autism (Carter, 2007), anxiety, and depression (Carter & Altemus, 2004), and possibly schizophrenia. Functional sex differences in neuropeptides, including AVP and possibly OT may have particular significance for understanding sexually biased disorders (Carter, 2007). Early manipulations of OT also can program various aspects of the body's management of stressful experiences, including measures of behavior, brain activity and chemistry, stress-related hormones, and even receptors for stress hormones in the heart (Pournajafi-Nazarloo *et al.*, 2007b).

REFERENCES

Bales, K. L., and Carter, C. S. (2003a). Sex differences and developmental effects of oxytocin on aggression and social behavior in prairie voles (*Microtus ochrogaster*). *Horm. Behav.* 44, 178–184.

Bales, K. L., and Carter, C. S. (2003b). Developmental exposure to oxytocin facilitates partner preferences in male prairie voles (*Microtus ochrogaster*). *Behav. Neurosci.* 117, 854–859.

Bales, K. L., Abdelnabi, M., Cushing, B. S., Ottinger, M. A., and Carter, C. S. (2004a). Effects of neonatal oxytocin manipulations on male

reproductive potential in prairie voles. *Physiol. Behav.* 81, 519–526.

Bales, K. L., Kim, A. J., Lewis-Reese, A. D., and Carter, C. S. (2004b). Both oxytocin and vasopressin may influence alloparental behavior in male prairie voles. *Horm. Behav.* 45, 354–361.

Bales, K. L., Pfeifer, L. A., and Carter, C. S. (2004c). Sex differences and effects of manipulations of oxytocin on alloparenting and anxiety in prairie voles. *Dev. Psychobiol.* 44, 123–131.

Bales, K. L., Kramer, K. M., Lewis-Reese, A. D., and Carter, C. S. (2006). Effects of stress on parental care are sexually dimorphic in prairie voles. *Physiol. Behav.* 87, 424–429.

Bales, K. L., Lewis-Reese, A. D., Pfeifer, L. A., Kramer, K. M., and Carter, C. S. (2007a). Early experience affects the traits of monogamy in a sexually dimorphic manner. *Dev. Psychobiol.* 49, 335–342.

Bales, K. L., Plotsky, P. M., Young, L. J., Lim, M. M., Grotte, N. D., Ferrer, E., and Carter, C. S. (2007b). Neonatal oxytocin manipulations have long-lasting, sexually dimorphic effects on vasopressin receptors. *Neuroscience* 144, 38–45.

Bales, K. L., Stone, A. I., Boone, E. M., and Carter, C. S. (2007c). Long-term and intergenerational effects of early handling on oxytocin, pair-bonding, and parenting in monogamous prairie voles. *Soc. Neurosci. Abst.* 209.15.

Bales, K. L., Van Westerhuyzen, J. A., Lewis-Reese, A. D., Grotte, N. D., Lanter, J. A., and Carter, C. S. (2007d). Oxytocin has dose-dependent developmental effects on pair-bonding and alloparental care in female prairie voles. *Horm. Behav.* 52, 274–279.

Barberis, C., and Tribollet, E. (1996). Vasopressin and oxytocin receptors in the central nervous system. *Crit. Rev. Neurobiol.* 10, 119–154.

Bester-Meredith, J. K., and Marler, C. A. (2003). Vasopressin and the transmission of paternal behavior across generations in mated, cross-fostered Peromyscus mice. *Behav. Neurosci.* 117, 455–463.

Bielsky, I. F., Hu, S.-B., and Young, L. J. (2005). Sexual dimorphism in the vasopressin system: Lack of an altered behavioral phenotype in female V1a receptor knockout mice. *Behav. Brain Res.* 164, 132–136.

Boone, E., Sanzenbacher, L. L., Carter, C. S., and Bales, K. L. (2006). Sexually dimorphic effects of early experience on alloparental care and adult social behaviors in voles. *Soc. Neurosci. Abst.* 578.6.

Carter, C. S. (1998). Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology* 23, 779–818.

Carter, C. S. (2003). Developmental consequences of oxytocin. *Physiol. Behav.* 79, 383–397.

Carter, C. S. (2007). Sex differences in oxytocin and vasopressin: Implications for autism spectrum disorders?. *Behav. Brain Res.* 176, 170–186.

Carter, C. S., and Altemus, M. (2004). Oxytocin, vasopressin, and depression. In *Current and Future Developments in Psychopharmacology* (J. A. den Boer, M. S. George, and G. J. ter Horst, Eds.), pp. 201–216.

Carter, C. S., and Keverne, E. B. (2002). The neuroendocrinology of social attachment and love. In *Hormones, Brain, and Behavior* (D. Pfaff, Ed.), Vol. 4, pp. 299–337.

Champagne, F., Diorio, J., Sharma, S., and Meaney, M. J. (2001). Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proc. Natl Acad. Sci. USA* 98, 12736–12741.

Champagne, F. A., and Meaney, M. J. (2006). Stress during gestation alters postpartum maternal care and the development of the offspring in a rodent model. *Biol. Psychiatr.* 59, 1227–1235.

Crepel, V., Aronov, D., Jorquera, I., Represa, A., Ben-Ari, Y., and Cossart, R. (2007). A parturition-associated nonsynaptic coherent activity pattern in the developing hippocampus. *Neuron* 54, 105–120.

Cushing, B. S., and Kramer, K. M. (2005). Mechanisms underlying epigenetic effects of early social experience: The role of neuropeptides and steroids. *Neurosci. Biobehav. Rev.* 29, 1085–1105.

Cushing, B. S., Okorie, U., and Young, L. J. (2003a). The effects of neonatal castration on the subsequent behavioural response to centrally administered arginine vasopressin and the expression of V1a receptors in adult male prairie voles. *J. Neuroendocrinol.* 15, 1021–1026.

Cushing, B. S., Yamamoto, Y., Hoffman, G. E., and Carter, C. S. (2003b). Central expression of c-Fos in neonatal male and female prairie voles in response to treatment with oxytocin. *Dev. Brain Res.* 143, 129–136.

De Vries, G. J., and Simerly, R. B. (2002). Anatomy, development, and function of sexually dimorphic neural circuits in the mammalian brain. In *Hormones, Brain, and Behavior* (D. Pfaff, Ed.), Vol. 4, pp. 137–192.

Diaz-Cabiale, Z., Olausson, H., Sohlstrom, A., Agnati, L. F., Narvaez, J. A., Uvnas-Moberg, K., and Fuxe, K. (2004). Long-term modulation by postnatal oxytocin of the alpha(2)-adrenoceptor agonist binding sites in central autonomic regions and the role of prenatal stress. *J. Neuroendocrinol.* 16, 183–190.

Francis, D., Diorio, J., Liu, D., and Meaney, M. J. (1999). Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science* 286, 1155–1158.

Francis, D. D., Young, L. J., Meaney, M. J., and {AQ3} Insel, T. R. (2002). Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: Gender differences. *J. Neuroendocrinol.* 14, 349–353.

Getz, L. L., and Carter, C. S. (1996). Prairie vole partnerships. *Am. Sci.* 84, 56–62.

Gimpl, G., and Fahrenholz, F. (2001). The oxytocin receptor system: Structure, function, and regulation. *Physiol. Rev.* 81, 629–683.

{AQ4} Glynn, L. M., Davis, E. P., Schetter, C. D., Chicz-Demet, A., Hobel, C. J., and Sandman, C. J. (2007). Postnatal maternal cortisol levels predict temperament in healthy breastfed infants. *Early Hum. Dev.*

Grosvenor, C. E., Picciano, M. T., and Baumrucker, C. R. (1993). Hormones and growth factors in milk. *Endo. Rev.* 14, 710–728.

Hammock, E. A., and Young, L. J. (2005). Microsatellite instability generates diversity in brain and sociobehavioral traits. *Science* 308, 1630–1634.

Husslein, P. (2002). Development and clinical experience with the new evidence-based tocolytic atosiban. *Acta Obstet. Gynecol. Scand.* 81, 633–641.

Insel, T. R., and Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc. Natl Acad. Sci. USA* 89, 5981–5985.

Kaffman, A., and Meaney, M. J. (2007). Neurodevelopmental sequelae of postnatal maternal care in rodents: Clinical and research implications of molecular insights. *J. Child Psychol. Psychiatr.* 48, 224–244.

Kimura, T., Saji, F., Nishimori, K., Ogita, K., Nakamura, H., Koyama, M., and Murata, Y. (2003). Molecular regulation of the oxytocin receptor in peripheral organs. *J. Mol. Endocrinol.* 30, 109–115.

Kramer, K. M., Cushing, B. S., and Carter, C. S. (2003). Developmental effects of oxytocin on stress response: Single versus repeated exposure. *Physiol. Behav.* 79, 775–782.

Kramer, K. M., Choe, C., Carter, C. S., and Cushing, B. S. (2006). Developmental effects of oxytocin on neural activation and neuropeptide release in response to social stimuli. *Horm. Behav.* 49, 206–214.

Kramer, K. M., Yoshida, S., Papademetriou, E., and Cushing, B. S. (2007). The organizational effects of oxytocin on the central expression of estrogen receptor alpha and oxytocin in adulthood. *BMC Neurosci.* 8, 71.

Leake, R. D., Weitzman, R. E., and Fisher, D. A. (1981). Oxytocin concentrations during the neonatal period. *Biol. Neonate* 39, 127–131.

Lim, M. M., Hammock, E. A. D., and Young, L. J. (2004). The role of vasopressin in the genetic and neural regulation of monogamy. *J. Neuroendocrinol.* 16, 325–332.

Mahalati, K., Okanoya, K., Witt, D. M., and Carter, C. S. (1991). Oxytocin inhibits male sexual behavior in prairie voles. *Pharmacol. Biochem. Behav.* 39, 219–222.

Marler, C. A., Bester-Meredith, J. K., and Trainor, B. C. (2003). Paternal behavior and aggression: Endocrine mechanisms and nongenomic transmission of behavior. In *Advances in the Study of Behavior*, Vol. 32, pp. 263–323.

Pedersen, C. A., and Boccia, M. L. (2002). Oxytocin links mothering received, mothering bestowed, and adult stress responses. *Stress* 5, 267.

Phelps, S. M., and Young, L. J. (2003). Extraordinary diversity in vasopressin (V1a) receptor distributions among wild prairie voles (*Microtus ochrogaster*): Patterns of variation and covariation. *J. Comp. Neurol.* 466, 564–576.

Porges, S. W. (1998). Love: An emergent property of the mammalian autonomic nervous system. *Psychoneuroendocrinology* 23, 837–861.

Pournajafi-Nazarloo, H., Carr, M. S., Papademetriou, E., Schmidt, J. V., and Cushing, B. S. (2007a). Oxytocin increases ER-alpha mRNA expression in the hypothalamus and hippocampus of neonatal female voles. *Neuropeptides*. 41, 39–44.

Pournajafi-Nazarloo, H., Perry, A., Papademetriou, E., Parloo, L., and Carter, C. S. (2007b). Neonatal oxytocin treatment modulates oxytocin receptor, atrial natriuretic peptide, nitric oxide synthase, and estrogen receptor mRNA expression in rat heart. *Peptides* 28, 1170–1177.

Stribley, J. M., and Carter, C. S. (1999). Developmental exposure to vasopressin increases aggression in adult prairie voles. *Proc. Natl Acad. Sci. USA* 96, 12601–12604.

Szyf, M., Weaver, I. C. G., Champagne, F. A., Diorio, J., and Meaney, M. J. (2005). Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Front. Neuroendocrinol.* 26, 162.

Tyzio, R., Cossart, R., Khalilov, I., Minlebaev, M., Hubner, C. A., Represa, A., Ben-Ari, Y., and Khazipov, R. (2006). Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science* 314, 1788–1792.

Wang, Z. X., Ferris, C. F., and De Vries, G. J. (1994). Role of septal vasopressin innervation in paternal behavior in prairie voles (*Microtus ochrogaster*). *Proc. Natl Acad. Sci. USA* 91, 400–404.

Wang, Z. X., Young, L. J., De Vries, G. J., and Insel, T. R. (1998). Voles and vasopressin: A review of molecular, cellular, and behavioral studies of pair bonding and paternal behaviors. *Prog. Brain Res.* 119, 483–499.

Williams, J. R., Catania, K. C., and Carter, C. S. (1992). Development of partner preferences in female prairie voles (*Microtus ochrogaster*): The role of social and sexual experience. *Horm. Behav.* 26, 339–349.

Williams, J. R., Insel, T. R., Harbaugh, C. R., and Carter, C. S. (1994). Oxytocin centrally administered facilitates formation of a partner preference in female prairie voles (*Microtus ochrogaster*). *J. Neuroendocrinol.* 247–250.

Winslow, J. T., Hastings, N., Carter, C. S., Harbaugh, C. R., and Insel, T. R. (1993). A role for central vasopressin in pair bonding in monogamous prairie voles. *Nature.* 365, 545–548.

Withuhn, T. F., Kramer, K. M., and Cushing, B. S. (2003). Early exposure to oxytocin affects the age of vaginal opening and first estrus in female rats. *Physiol. Behav.* 80, 135–138.

Witt, D. M., Carter, C. S., and Insel, T. R. (1991). Oxytocin receptor binding in female prairie voles – Endogenous and exogenous estradiol stimulation. *J. Neuroendocrinol.* 3, 155–161.

Yamamoto, Y., Cushing, B. S., Kramer, K. M., Epperson, P. D., Hoffman, G. E., and Carter, C. S. (2004). Neonatal manipulations of oxytocin alter expression of oxytocin and vasopressin immunoreactive cells in the paraventricular nucleus of the hypothalamus in a gender specific manner. *Neuroscience* 125, 947–955.

Yamamoto, Y., Carter, C. S., and Cushing, B. S. (2006). Neonatal manipulation of oxytocin affects expression of estrogen receptor alpha. *Neuroscience* 137, 157–164.

Young, E., Carter, C. S., Cushing, B. S., and Caldwell, J. D. (2005a). Neonatal manipulations of oxytocin alter oxytocin levels in the pituitary of adult rats. *Horm. Metabol. Res.* 37, 397–401.

Young, L. J., Murphy Young, A. Z., and Hammock, E. A. (2005b). Anatomy and neurochemistry of the pair bond. *J. Comp. Neurol.* 493, 51–57.

Author Queries

{AQ1} Please update the reference Tyler, Carter, and Bales and add to the references list.
{AQ2} Please specify 2004a, or 200b, or 2004c for the reference Bales et al. (2004) cross-referred here.
{AQ3} Please confirm the page range for the reference Francis *et al.* (2002).
{AQ4} Please provide volume and page numbers for the reference Glynn *et al.* (2007).
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