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Developmental consequences of oxytocin

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Abstract

This paper examines the developmental effects of the mammalian neuropeptide, oxytocin (OT). In adults, OT is the most abundant neuropeptide in the hypothalamus and serves integrative functions, coordinating behavioral and physiological processes. For example, OT has been implicated in parturition, lactation, maternal behavior and pair bond formation. In addition, OT is capable of moderating behavioral responses to various stressors as well as the reactivity of the hypothalamic–pituitary–adrenal (HPA) axis. Neonates may be exposed to hormones of maternal origin, possibly including peptides administered to the mother in the perinatal period to hasten or delay birth and in milk; however, whether peptide hormones from the mother influence the developing infant remains to be determined. In rodents, endogenous OT is first synthesized during the early postnatal period, although its functions at this time are not well known. Experiments in neonatal prairie voles have documented the capacity of OT and OT receptor antagonists to have immediate and lifelong consequences for social behaviors, including adult pair bonding and parental behaviors, as well as the reactivity of the HPA axis; most of these effects are sexually dimorphic. Possible mechanisms for such effects, including long-lasting changes in OT and vasopressin, are summarized.

Keywords: Oxytocin; Vasopressin; Development; Sex differences; Parental behavior; Pair bonding; Stress; Prairie voles

1. Introduction

Developmental processes are designed to be changed. The survival of each species and each individual member of a species depends on the ability to adapt to both immediate and long-term demands of the environment. The long-term messengers of change are genes, and the development of the mammalian nervous system and its functions reflects a species-typical genetic code. Gene expression in turn is regulated by epigenetic and experiential factors. Individual experiences must be transformed into long-lasting neural adaptations and subsequent patterns of anatomy, physiology and behavior [68].

Among the epigenetic processes capable of mediating long-lasting changes in anatomy and physiology are neuroendocrine systems described in this paper. In mammals, the mother–infant interaction and other aspects of the early postnatal period may have profound behavioral effects; these effects in turn may produce long-lasting changes in neuroanatomy and neuroendocrinology [61,64,67,72]. Physiological and anatomical processes responsible for individual adaptation may enhance survival, but the susceptibility of these systems to change also leaves these same systems vulnerable to modifications that in a later context may be maladaptive.

Peptide hormones and their receptors have properties that identify these systems as candidate substrates for the transduction of early experiences into both short-term and long-term behavioral changes. One goal of the present review is to survey the role of oxytocin (OT) in neural and behavioral development. Research directly relevant to this question is limited. However, a variety of experiences including the birth process, breast-feeding and other aspects of parent–infant interactions can influence peptide systems during mammalian development. Such experiences have the potential to reorganize brain function and behavior. An additional purpose of this paper is to draw on recent findings from animal research to question possible long-term consequences of such manipulations for behavior and physiology, especially in the context of human development.

OT has a host of functions in both females and males [19,42,53,86,112,113]. Many aspects of medicine and child rearing involve changes in exogenous or endogenous OT and thus have the potential to influence these systems, including the reproductive, cardiovascular and immune

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systems. Using animal models, it is possible to identify and characterize targets that may be vulnerable to long-lasting effects of developmental manipulations of OT. The list of tissues sensitive to the developmental effects of OT of course extends beyond the nervous system [82]. Animal research, including recent in vitro studies, suggests insights into the behavioral and physiological consequences of OT and directions for human research.

Manipulations of peptides, particularly OT, during the perinatal period have become an accepted—but largely unstudied—aspect of human development. For example, synthetic OT (pitocin) is used to induce or hasten childbirth [34,108]. In cases of premature delivery, OT antagonists (OTA) are capable of slowing or preventing labor [47]. Further complicating the peptide history of human infants is the fact that during childbirth women and potentially their infants may be exposed to varying amounts of exogenous and endogenous hormone, analgesics, anesthesia, the stressful side effects of the birth experience and in some cases surgery. Although such complex manipulations are routine in modern obstetrics, little is known regarding the possible consequences of such treatments. In addition, child rearing procedures, such as the degree to which an infant is held or touched or the presence or absence of breast-feeding, may indirectly affect the peptide experience and subsequent physiology and behavior of the infant [113]. Animal models provide an opportunity to examine the effects of peptides at mechanistic levels that are impossible in humans while generating hypotheses that can inform subsequent investigations of the role and mechanisms through which hormones influence physiology and behavior.

2. OT and vasopressin

OT and the related hormone, arginine vasopressin (AVP), are small nonapeptide hormones that differ from each other in only two of nine amino acids. Both are considered uniquely mammalian hormones, although OT also is found in certain primitive fish, probably as the result of an independent evolutionary event [1]. Genes for vasopressin-like and OT-like peptides have been identified in mollusks, and van Kesteren et al. [116] suggest that prohormones of the vasopressin–OT superfamily were present in the common ancestors of vertebrates and invertebrates (Tables 1 and 2).

OT was first identified as a reproductive hormone with a major role in childbirth (uterine contractions) and lactation (milk ejection) [53,95,96]. Vasopressin's functions were initially described in the context of cardiovascular functions (especially blood pressure) and water regulation by the kidney; vasopressin is also known as the ''antidiuretic hormone'' or ADH [7].

In rats, OT is the most abundant neuropeptide (based on mRNA expression) in the hypothalamus [39]. OT is produced in high concentrations in specific hypothalamic nuclei, including the supraoptic nucleus (SON) and paraventricular nucleus (PVN). OT and vasopressin are transported from these source nuclei to the mammalian posterior pituitary by neurosecretion [42]. Although the brain is the major source of OT, other tissues, including the uterus, gonads, heart and thymus, can synthesize this hormone at levels that are functionally significant.

Table 1
Features of the neurobiology of OT and AVP (see text for details and exceptions)

Ancient origins prior to the separation between vertebrates and invertebrate [116]
Specific nine-amino acid structures of OT and AVP are novel to mammals [1]
OT is most abundant neuropeptide in the hypothalamus as indexed by mRNA [39]
Synthesized in largest cells in the CNS (magnocellular neurons) as well as in other smaller cells [42]
Transported by neurosecretion to posterior pituitary but also released in CNS [42]
Sibling hormones, OT and AVP, have consequences for each other's functions [15,35,42]
OT has only one known receptor, but may bind to AVP receptors [42,81]

Various types of stimuli associated with social interactions can release OT. OT is secreted in pulses during lactation by breast stimulation and can become conditioned to stimuli associated with the infant [113]. OT is released by genital stimulation and during sexual behavior in a variety of species [14]. In rats, nongenital tactile contact releases OT, even in anesthetized animals. In addition, in both male and female rats, OT is released following immersion in warm water and by vibration, electroacupuncture, tactile stimulation and massage [102,114].

Both OT and vasopressin are released within and have receptors in the nervous system [6,42]. Receptors for both OT and vasopressin are localized in areas of the nervous system, especially in the brainstem, which play a role in reproductive, social and adaptive behaviors [50,74,122,128] and the regulation of the autonomic nervous system [89,97].

In contrast to most biologically active compounds, OT appears to have only one form of receptor. For example, vasopressin has at least three distinct receptors [81]. The novel neuroanatomy of the oxytocinergic system allows a coordinated effect on behavior, autonomic functions and peripheral tissue.

In some but not all cases, vasopressin and OT have opposite functions, possibly because they are capable of acting as antagonists to each other's receptors [23,31,35]. For example, both hormones have been implicated in the control of the autonomic nervous system, with OT having primarily parasympathetic actions and vasopressin serving as a central and peripheral component of the sympathetic nervous system [89]. The behavioral effects of OT and vasopressin correlate with their autonomic actions, supporting the hypothesis that OT and its vagal/parasympathetic activities may integrate a variety of metabolic and behavioral systems [113]. Thus, the release of OT or treatment with this peptide is associated with a reduction in anxiety,

Table 2
OT is associated with experiences or conditions that facilitate social bond formation

Conditions associated with	Release of endogenous OT	Facilitated by exogenous OT	Associated with social bond formation
Birth	+	+	+
Lactation	+	+	+
Parental behavior (female)	+	+	+
Sexual behavior	+	+	+
Stress responses[a]	+/−	+	+
Coping with stress[b]	+	+	+[a]

[a] The behavioral and endocrine responses to stressors tend to be species and gender specific. For example, in rats, acute stress releases OT, while in humans this may not be the case (reviewed in Refs. [15,16]). Pairing with a novel stranger, a stressor in rats and mice, is associated with an immediate decline in corticosterone in prairie voles.

[b] In female prairie voles, even a brief stressful experience or corticosterone treatment can inhibit female–male pair bonding; however, female–female social bonds were facilitated by exposure to a stressor [15,26,27]. In male prairie voles, acute stress or corticosterone treatment facilitates the onset of pair bonding. In both male and female prairie voles, exogenous AVP as well as OT can facilitate heterosexual pair bonding. However, endogenous AVP, which is androgen dependent in the limbic system, may be more prevalent in males than females, allowing males to form new heterosexual pair bonds in the presence of stressors.

obsessiveness and stress reactivity and may serve to counteract the defensive behavioral strategies associated with stressful experiences and the central release of vasopressin and other peptides, such as corticotropin-releasing hormone (CRH) [15,16].

Plasticity and species and individual variability are characteristics of the receptors for both OT and vasopressin [128]. Even the distribution of neuropeptide receptors is not static across the life span; e.g., in rats, OT receptors are abundant in the neonatal cortex and decline to low levels in adulthood [110,118]. In contrast, adult prairie voles retain the capacity to produce OT receptors in the cortex [123]. In addition, the hypothalamic neurons that produce OT have a novel degree of plasticity, even in adulthood [107]. For example, within the SON and PVN, changes in the morphology of OT neurons and associated glia occur during parturition and lactation; within a matter of hours, stimulation of this system can lead to an increase in the number of synapses, which may in turn facilitate the pulsatile firing patterns that are characteristic of OT neurons.

3. Evidence from "knockout" mice

One approach to understanding the functions of a peptide such as OT is the study of animals that lack the gene for this peptide [28,51]. Natural mutations of OT have not been reported, possibly because these mutations can affect reproduction and thus require special rearing procedures. However, information regarding the functions of OT may be derived from the study of mice that are homozygous for deletion of exon 1 of the OT gene, containing the OT nonapeptide sequence. These genetic experiments are thus far only possible in mice (also known as "OT knockout mice, OTKO"). OTKO mice did reach sexual maturity, showed sexual behavior and subsequently delivered offspring, which they retrieved and treated normally. However, the OTKO mice were incapable of milk ejection and thus were unable to rear their own young [78]. These experiments challenge the essential role of OT in several mammalian functions, including birth. In spite of the widespread use of OT to assist or hasten delivery in humans, there is evidence, at least for mice, that other neurochemical processes can substitute for OT in its absence. Furthermore, as discussed in detail by Russell and Leng [95], there is "much stronger evidence that OT plays an important role in the progress of labor in many species" and OTAs are capable of decreasing uterine contractile force. In addition, unlike the rat or human, the uterus of the pregnant mouse does not express the OT gene.

Laboratory mice, including those used in the OTKO experiments, show spontaneous maternal responsiveness, even in virgin females or pregnant females that have not yet given birth. Thus, OT released during birth probably has little effect on maternal behavior in mice. In rats, there is ample evidence that OT can play a role in both the initiation and the maintenance of maternal behavior, although again other processes may substitute for OT [86]. In contrast to the apparent redundancy of the processes responsible for birth or maternal behavior, milk ejection (perhaps in all mammals) does rely on OT and thus was absent in the OTKO mice. Russell and Leng [95] suggest that "this may reflect the fact that lactation, or specifically milk ejection, is recent in evolution, in contrast with egg laying, the precursor of parturition," and state that the "neurohypophysial system regulates the delivery of progeny in all vertebrates, and during its long evolutionary history, other mechanisms may have evolved convergent roles." Quantitative studies of the behavior of OTKO mice are in progress in several laboratories and there is increasing evidence that OT has regulatory effects on both social behaviors and reproduction [29,51,52]. Based on data from existing studies, it seems that OT plays a role in many mammalian functions, although it does not act alone, and (with the exception of milk ejection) many aspects of OT's effects may continue in the absence of the hormone. In some cases, as reviewed by Russell and Leng [95], it is possible that vasopressin (which is still produced by OTKO mice) can assume some of the functions of OT. In addition, mechanisms that involve neither OT nor vasopressin may exist, providing redundancy in many mammalian systems. Such redundancy is common in biological systems and provides a substrate for the natural variance necessary for individual adaptation and species survival.

From an experimental point of view, genetically manipulated animals have the advantage of being well

defined at the molecular level. However, because the deficits of the OTKO mice are lifelong, studies of OTKO animals involve changes throughout the life span and do not specifically address the early or developmental effects of OT.

4. Developmental effects of neuropeptides in rats

In rats, genes for OT and vasopressin are transcribed as early as day 16 of intrauterine life. Vasopressin, measured by immunohistochemistry, also is first detected in the SON on day 16 of gestation. However, OT levels are low during gestation, and synthesis of OT is first detected by immunohistochemistry on the second day of postnatal life; these findings suggest that the gene encoding OT is regulated at the posttranscriptional level [65]. The OT receptor also first appears in the postnatal period [101]. Thus, the production of OT and possibly its receptor as well [22] may be especially vulnerable to postnatal experience.

Research first described in rats reveals that peptide manipulations during development [9–11,104] can alter the sensitivity of the adult nervous system to subsequent hormonal experiences, termed by Czaba [24] "hormonal imprinting." There is increasing evidence for tuning or programming of neuronal systems by early experiences, in some cases by endogenous or exogenous hormones. For example, because of the interdependence of steroid and peptide hormones, developmental changes associated with perinatal stress or gender-dependent androgenization–estrogenization may alter the subsequent sensitivity of central system to peptides.

Early experience, including maternal stimulation, has lifelong consequences for the behavior and physiology of animals [8,64,72]. For example, high levels of maternal stimulation can both down-regulate the hypothalamic–pituitary–adrenal (HPA) axis and affect gene expression for CRH and vasopressin or their receptors. There is some evidence that OT expression in rats is up-regulated by maternal licking and grooming, possibly playing a role in both later behavior and HPA reactivity [22]. In female but not male rats, adult OT receptor binding was increased in animals that had received high levels of maternal stimulation during their infancy [38].

In another example of the possible effects of early neuroendocrine manipulations, male rats treated with vasopressin during the first week of life had in adulthood lower levels of OT receptor gene expression in the PVN of the hypothalamus [81,115]. This finding is of particular relevance because stressful experiences have the potential to release vasopressin in early life and because the PVN is a major neural site for the integration of autonomic and neuroendocrine processes.

There is also evidence that OT manipulations in early life change the response of an infant to its mother. OT injections, given peripherally to the mother, can facilitate nipple attachment by young rat pups [99]. Rat pups also show preferences for specific odors that are associated with exposure to their mothers. Preferences for the mother do not develop in animals that are pretreated with an OTA [76]. OT injections can produce rapid effects on the tendency of infants to cry [48]. Thus, OT may act on both the mother and the infant to influence the response of young animals to their maternal environment.

5. Prairie voles as a model for the analysis of developmental effects of neuropeptides

Prairie voles (*Microtus ochrogaster*) are the subjects in a series of ongoing experiments on the developmental effects of OT. Adult prairie voles are behaviorally sensitive to OT [18,23,50] and have comparatively high blood levels of OT (Kramer et al., unpublished data). Prairie voles also spontaneously exhibit several traits found in humans, including the capacity to develop adult heterosexual pair bonds and for both parents and other family members to show parental care [19,20,92]. Peptides have been implicated in each of these traits.

Several other behavioral systems and physiological processes, including social and reproductive behaviors and the regulation of the HPA axis, are modulated by social behaviors and/or OT in adulthood [15,77,117,120]. These processes have been targeted as likely candidates for developmental plasticity. The purpose of ongoing research in my laboratory is to examine the hypothesis that developmental manipulations of OT can influence various endocrine and behavioral systems and that these effects may be detected in later life.

5.1. Mating systems and social behavior

Prairie voles are most readily understood in the context of their social systems and natural history [17,20]. One common method for categorizing species is based on mating systems, characterized by the presumed choice or number of sexual partners, and often focused on male behavior. The most common mating system in mammals is called polygamy (many mates) or polygyny (many female mates). The less common alternative, monogamy (one mate), is estimated to occur in about 3% of mammalian species [57], while polyandry (many male mates) is even rarer.

Prairie voles are small arvicoline rodents found in grasslands throughout Midwestern North America. Prairie voles exhibit all of the features of social monogamy, including the formation of pair bonds between adult conspecifics, biparental care and alloparental behavior. This species has attracted particular interest because they can be studied under both field and laboratory conditions. Field studies begun by Getz et al. provided evidence that prairie voles

(studied in central Illinois) form lifelong social bonds [40,41,71]. Male and female pairs of this species maintain a common nest and territory and tend to enter live traps together as long as both members of the pair are alive. The degree to which prairie voles are sexually monogamous in nature is unknown; however, laboratory and field observations do not support the assumption that sexual exclusivity is a necessary component of the social system of this species [17]. By contrast, in less social, typically polygamous meadow voles (*Microtus pennysylvanicus*) and montane voles (*Microtus montanus*), males and females have separate nests and territories and are usually trapped alone [40,41,71]. These species differences are not absolute. For example, there also are indications that prairie voles captured in other habitats, such as the less resource-abundant environment of eastern Kansas, may show fewer of the traits of monogamy [20,93]. In addition, when reared under winter-like photoperiods, meadow voles may form pair bonds and show biparental behavior [83,84]. Nonetheless, under comparable conditions, species that are not socially monogamous are less likely than prairie voles to engage in social contact and less likely to exhibit selective partner preferences [19,32].

Prairie voles in central Illinois live in nature in communal family groups comprised primarily of a male and female pair and their (presumptive) offspring. About 70–75% of young voles do not leave their natal family [41,71]. The original breeding pair within a family has a reproductive advantage, while most other members of the communal groups are reproductively inactive offspring. These offspring serve as helpers at the nest or alloparents, presumably gaining reproductive advantages in part through inclusive fitness. Familiarity inhibits reproduction in young prairie voles, and incest is avoided through several mechanisms, including reluctance to mate with a family member. Young males remain sexually suppressed within the family nest and must leave the family group to reproduce [20]. New pairs are most likely to form when previously naïve males and females leave their group, meet an unfamiliar member of the opposite sex, develop new pair bonds, mate and generate their own families.

It is possible to influence pair bonding by a variety of hormonal manipulations [17,20,50]. Pair-bonded males become highly aggressive following mating and probably patrol the runways that lead to their nest. If successful in defending his partner, then the male may father the offspring that his partner delivers. In nature, when one member of the pair dies or abandons the nest, fewer than 20% of the remaining partners form a new pair bond [41]; thus, for most prairie voles, pair bonds last until the pair is separated by the death of one partner.

Enhanced sociality and parental behavior follows treatment with OT in adult animals. Previous research [23,122,124] has shown in adult prairie voles as well as other species [125] that OT can facilitate social contact. This finding may generalize to the adult–infant interaction as well. Maternal behavior increases in female rats [87] and in sheep receiving CNS injections of OT [55,56]. The capacity of OT to facilitate maternal behavior has recently been replicated in female prairie voles (Bales and Carter, unpublished data [5]). Reproductively naïve female prairie voles treated with OT were approximately twice as likely to show maternal behavior as females receiving a control injection. This effect was blocked by simultaneous treatment with an OTA. Treatment with vasopressin did not significantly facilitate maternal behavior in female prairie voles. Male prairie voles show a high level of spontaneous parental behavior, and in our laboratory, additional exogenous OT or vasopressin did not further enhance parental behavior. In addition, males given a single treatment with an antagonist for either the OT (OTA) or the vasopressin (AVPA-V1a) receptor continued to show paternal behavior. However, a combined treatment with OTA/AVPA-V1a was capable of inhibiting spontaneous paternal behavior, suggesting that OT and vasopressin may both be involved in male parental care in prairie voles (Bales and Carter, unpublished data).

5.2. Selective social behavior—a role for OT

Sociality as well as aggression can be either nonselective or selective. Selective aggression or mate guarding has been used as one index of social bonds. OT (intracerebroventricular in adults) increases the tendency to show both selective and nonselective social behaviors [23,122,124].

In prairie voles, sexual experience can hasten the onset of selective partner preferences [119] and in males induces aggression toward strangers [121]. It is also known that OT can be released during mating (reviewed in Refs. [2,14]). These observations suggested that OT might promote social bonding. OT infusions, when centrally administered using several different methods, did indeed facilitate the onset of partner preferences in sexually naïve female and male prairie voles [23,49,120]. Moreover, treatment with a selective OTA reduced the behavioral effects of exogenous OT [23] and also blocked partner preference formation during prolonged cohabitation in females [120]. However, as with male parental behavior, the presence of both OT and vasopressin may be necessary for the full expression of a partner preference, especially in males.

In addition, in male prairie voles, vasopressin facilitates the onset of stranger-directed aggression. Experimental evidence for a role of vasopressin in males comes from the fact that pretreatment with a AVPA-V1a receptor antagonist inhibited the selective social behavior and aggression that normally results from sexual experience [121]. In addition, exogenous vasopressin administration facilitates the onset of selective aggression in this species. It is likely that interactions between vasopressin and OT play a role in many of the effects of OT [23,35]; however,

Table 3
Shared properties between prairie voles and the postpartum period in human females

Prairie vole (species traits)	Postpartum female human
Capacity to form pair bonds	Capacity to form maternal–infant bonds
High levels of OT	High levels of OT (parturition and lactation)
Low gonadal steroids	Gonadal hormones suppressed, especially in lactating females
Glucocorticoids high and decline during heterosexual pairing	Glucocorticoids high in pregnancy and parturition but reduced in lactation

a detailed analysis of the behavioral effects of vasopressin is beyond the scope of the present review.

It is interesting to note that several characteristics of prairie vole biology are shared by postpartum women. In both cases, social bonds may form with ease (Table 3).

6. Developmental consequences of manipulations of OT in prairie voles

In ongoing research, we are examining the effects in infant prairie voles of intraperitoneal injection, within 24 h of birth (postnatal day 1), with either (a) 3 μg of OT, (b) 0.3 μg of OTA or (c) isotonic saline vehicle control (SAL) or in control infants that are handled without injection (HAN) (Table 4). The OTA used in these studies binds to the OT receptor but also can affect the vasopressin receptor [69]. This OTA has been used extensively in behavioral work and the properties of the OTA used in these studies are somewhat similar to those of the clinically available OTA, Atosiban. Atosiban is prescribed to prevent or stop contractions during premature labor [47]. (More selective receptor antagonists for OT are now available, but less is known regarding their behavioral effects [69,70].) The first step in these studies was to document the ability of neonatally injected peptides to influence the infant's nervous system. In parallel studies, we have also examined the long-term behavioral consequences of neonatal manipulations of OT or OTA on social and reproductive behaviors as well as corticosterone levels prior to or following a stressor.

6.1. c-Fos

Through measurement of c-Fos protein, it is possible to compare neural activation in defined areas of the CNS as a function of neonatal manipulations. Following the protocol described above, neonatal treatments were administered on postnatal day 1; 1 h later, brain tissue was processed for c-Fos immunoreactivity (c-Fos-IR) (Cushing et al., unpublished data). c-Fos-IR was detected in the SON and PVN as well as in other hypothalamic and thalamic nuclei. In the SON, the basal expression of c-Fos-IR in controls (HAN or SAL) was sexually dimorphic, with comparatively high levels of activity in control females but not in control males. In males injected with OT, and to lesser extent after OTA, there was an increase in c-Fos-IR in the SON, producing a c-Fos pattern in OT-treated males that was similar to that seen in untreated females.

6.2. OT and vasopressin immunoreactivity

Another histological study of animals treated with OT has revealed long-lasting increases in immunoreactive OT (OT-IR) and vasopressin (AVP-IR) (Ref. [126] and Yamamoto et al., unpublished data). Females treated with OT on day 1 postpartum showed increased OT-IR in the PVN (especially in the magnocellular region) when studied on day 21; this change was not seen in males or the SON. However, males exposed to the OTA on day 1 and sacrificed on day 21 showed a reduction in AVP-IR in the magnocellular PVN, with no change in OT-IR in the PVN. Because the magnocellular region of the PVN is an important site for the synthesis of OT and vasopressin, these findings support the hypothesis that early manipu-

Table 4
Long-term consequences of neonatal manipulations of OT or OTA in male prairie voles

Responses when tested in later life	Neonatal saline	Neonatal OT	Neonatal OTA[a]
Parental behavior[b]	Normal-high	Normal-high	Low
Partner preferences[b]		Facilitated	
Male–male aggression[b]	Moderate	Moderate	Low
Corticosterone-basal	Normal	Normal	High
Corticosterone after swim	Normal	Normal-low	Atypical
OT-IR in PVN (day 21)[c]	Normal	Normal	Normal
AVP-IR in PVN (day 21)[c]	Normal	Normal	Reduced

[a] The OTA used in this study is capable of binding to both OT and AVP receptors [69,70].

[b] In adult male prairie voles, these behaviors are regulated in part by AVP. Treatment on day 1 postpartum with OTA was followed in later life by reductions in spontaneous parental behavior (alloparenting) as well as an increased tendency to attack pups [88]. These males also showed lower levels of aggression than controls or OT-treated males [3] and atypical corticosterone responses to a 3 min swim stress (Bales et al., unpublished data). In adult male prairie voles, both AVP and OT may play a role in the onset of partner preferences. Adult males were paired with a female partner for 1 h followed by a test for partner preferences. Only males exposed to OT on day 1 postpartum showed a significant partner preference after 1 h of pairing, while both saline- and OTA-treated males were equally likely to prefer either a familiar partner or a stranger [4].

[c] In a study of animals receiving neonatal treatments on postpartum day 1 that were later studied on day 21, PVN tissue from neonatally OTA-treated males revealed reductions in AVP-IR but normal levels of OT-IR. Females showed increased OT-IR after either OT or OTA and no effect of neonatal treatments on AVP-IR (Yamamoto et al., unpublished data and Ref. [126]).

lations of OT might influence subsequent production of this peptide and that these effects are in some cases gender specific. We also have begun to examine the parameters of possible behavioral effects of early OT or the OTA used in this study.

6.3. Parental behavior

At 21 days of age, animals treated on day 1 postpartum with OT or OTA were tested for spontaneous parental (alloparental) behavior. An animal was considered "alloparental" if it huddled over or retrieved an infant. Males from the control groups or receiving neonatal OT showed high levels of spontaneous parental behavior, and pup-directed attacks were rare. In contrast, males treated with OTA on the first day of life showed low levels of parental behavior and were more likely to attack infants than any other group. Females were less likely than males to be spontaneously parental. However, pup attacks by females were rare and the neonatal treatments used here did not significantly increase or decrease female alloparental behavior or pup-directed aggression [88].

6.4. Partner preferences/selective sociality

At 60–90 days of age, males and females from neonatally OT-manipulated groups were tested for selective partner preferences, which is a component of pair bonding. Animals undergoing the partner preference test received 1 h of cohabitation with an unrelated potential "partner" of the opposite sex. Based on earlier research, we did not expect that 1 h would be long enough to allow the formation of a partner preference [25,119]. Experimental animals then were placed in a test apparatus, which allowed a choice between the familiar animal (i.e., partner), a stranger or an empty cage in a 3 h test. A partner preference was defined by a significant difference in social contact with the partner versus the stranger [119]. Selective aggression, possibly for the purpose of mate guarding, is another aspect of pair bonding [19]. This is most commonly assessed by testing animals in a dyadic encounter with a member of the same sex. In the present study, aggression was assessed before and again after each animal was tested for a partner preference.

6.5. Sex differences

Among males, OT-treated animals were the only group that formed a significant partner preference [4]. In contrast, saline- and OTA-treated males did not show any tendency to form a partner preference under these test conditions, while unhandled control males showed a nonsignificant tendency toward a preference for the familiar partner but did not differ significantly from either OT-, OTA- or saline-treated animals. In these males, aggression did not differ among the four groups. Females from the four groups listed above also were tested for partner preferences after only 1 h of cohabitation; in this experiment, females from all groups tended to show a preference for the familiar partner. Neonatal OTA did not inhibit the later development of female partner preferences. Because of the unexpectedly rapid formation of a partner preference in females in all groups, any possible increases in the positive social behaviors of OT-treated females could not be detected. However, indices of intrasexual aggression did provide evidence that early OT was behaviorally active in these females. When tested as adults for female–female aggression after male exposure, females treated with OT on day 1 postpartum were more aggressive and less likely to engage in social contact with other females [3]. We have previously found that in untreated prairie voles female–female aggression is stimulated by cohabitation with a male; however, in normal females, a period of several days, rather than 4 h as used here, is necessary for the induction of aggression [12]. Thus, a rapid onset of intrasexual aggression could be interpreted as a facilitation of mate guarding in neonatally OT-treated females.

6.6. HPA axis activity

There is evidence that OT plays a role in the regulation of the HPA axis in adults of this species [15]. In addition, HPA axis reactivity to social stimuli may be influenced by the emotional state or history of an animal. For these reasons, we examined the possibility that early OT or OTA might alter HPA activity (Kramer, Bales and Carter, unpublished data). Blood was sampled in animals 1 week prior to and again 30 min after a 3 min swim challenge. OTA-treated males showed higher baseline levels of corticosterone (significantly higher than those in handled only animals) and atypical responses to stress. For example, in OTA-treated animals, the increase in corticosterone that typically follows a stressor was not observed [26,27,106], and some animals even showed lower levels of corticosterone in the postswim sample. The individual variation in corticosterone levels was especially high in OTA-treated males, and the failure to shown an increase in corticosterone after swimming was not limited to animals with high basal corticosterone levels. In females, basal or swim-induced changes in corticosterone did not differ among neonatally treated groups.

The time course of HPA axis activity and other hormones, including ACTH, AVP and CRH [15,28], remains to be measured in these animals. However, it seems likely that early manipulations of OT or OTA can produce long-term changes in reactivity to social and physical stimuli. These findings have at least superficial resemblance to disorders, such as posttraumatic stress disorder (PTSD), which are characterized by emotional hyperresponsivity to social stimuli and atypical HPA axis responses, in some cases lower than usual, to various stressors [127].

7. Mechanisms of interspecific and intraspecific variation

The neurochemicals that have been implicated in social behavior, including OT, are structurally identical among mammalian species. Whether the relative abundance of these peptides plays a role in the development or expression of the traits of social monogamy is not easily determined. However, in adulthood, prairie voles do have high blood levels of OT, at least twice the levels measured in rats (Kramer et al., unpublished data). Bonnet macaque monkeys, which are social, have higher CSF levels of OT and lower levels of CRH when compared with less gregarious and more aggressive pigtail macaques [91]. However, evidence linking blood or CSF levels of peptides to behavior is still correlational and thus indirect.

7.1. Species-typical patterns of HPA axis activity and reactivity

Prairie voles, along with a number of other social monogamous species, exhibit the traits of glucocorticoid resistance, including in adulthood high basal levels of corticosterone, ACTH and glucocorticoid receptor insensitivity [106]. However, the glucocorticoid levels of prairie voles in the postnatal period are not exceptionally high (Kramer, unpublished data). The endocrine factors responsible for this shift from neonatal to adult patterns of HPA axis activity in prairie voles are under investigation in my laboratory. Preliminary studies have revealed that animals exposed to OTA on the first day of life are, as early as day 8 postpartum, already unusually reactive to the stress of separation; this effect also was sexually dimorphic, but in this case females were more affected than males (Kramer, unpublished data). These findings suggest a role for OT in the development of species-typical patterns of HPA axis reactivity. Endocrine (HPA) and emotional reactivity could influence the tendency of an individual to exhibit social behaviors toward its own young or the offspring of others [67]. Atypical endocrine responses to stressors, such as those seen in animals exposed to neonatal OTA, also could have important implications for parental and other social behaviors.

7.2. Developmental effects of peptides

During development, exposure to peptides and steroids may reprogram the nervous system, altering thresholds for sociality, emotionality and aggression. Among the likely sources of interspecific and intraspecific variation with relevance to behavior are CNS peptide receptors [123,128]. Species-typical variations in peptide receptors are apparent in early development [118]. It has been suggested that early exposure to a particular peptide can alter the subsequent development of receptors for or sensitivity to that peptide, termed ‘‘hormonal imprinting’’ [24]. Developmental exposure to a particular peptide also has the potential to alter the expression of receptors for other peptides. For example, there is in rats evidence that early exposure to vasopressin can inhibit gene expression for OT receptors in the PVN [81,115]. Additionally, in rats, there is evidence that neonatal stress can influence hippocampal OT receptors [80].

Young [128] has compared the genes for OT receptors in prairie voles and montane voles and found that these receptors are ‘‘virtually identical’’ in genetic structure in these species. However, promoter elements can regulate the expression of these receptor genes in particular tissues; subtle but potentially important species differences in base sequences could play a role in interspecific variations in peptide receptor distributions.

7.3. Developmental effects of steroids

One of the characteristics of socially monogamous mammals is a comparative lack of sexual dimorphism in physical appearance; it can be difficult to distinguish males from females of monogamous species by external appearance or body size [32,57]. Androgenic steroids, including testosterone and especially dihydrotestosterone, typically regulate the development of masculine genitalia [43] and may play a role in male–female differences in body size. Thus, the apparent genital feminization, which is a trait of monogamous species, could reflect a comparative dearth of androgens and/or an insensitivity to endogenous androgen or its metabolites during development. Androgen levels measured in blood in monogamous voles do tend to be lower than those in nonmonogamous species [58]. At least among vole species, the absence of physical sexual dimorphism in monogamous species may reflect a comparative absence of or insensitivity to sex steroids, especially in early life [19].

It is possible that high levels of social behavior, including the tendency to form pair bonds and biparental care, also emerge in the relative absence of developmental exposure to high levels of androgens. Male parental and alloparental behavior are traits of some, but not all, pair bonding species. There is evidence from a variety of species that exposure to androgens in early life, and in some species in adulthood, can inhibit the tendency to be parental. However, gonadal steroids in prairie voles are not without physiological consequences. For example, recent research in prairie voles has revealed that castration on day 1 postpartum is followed in later life by an increase in hypothalamic estrogen receptors (Cushing, unpublished data). In addition, exposure to steroids, including testosterone or corticosterone during perinatal life, can reduce the tendency of young prairie voles to prefer a sibling, while increasing the chances that treated animals will prefer a novel stranger [92]. The presence or absence of androgens at various times in the life span also may play a role in the development of the capacity to respond to peptides in adulthood. Vasopressin is

capable of facilitating pair bond formation in male prairie voles [23,121]. However, when males are castrated on the first day of life, they are, as adults, less sensitive to the behavioral effects of vasopressin; this insensitivity also might be related to castration-related reductions in estrogen receptors in this species (Cushing and Okorie, unpublished data).

In the relative absence of high levels of androgens, some species may rely more directly on neuropeptides, including vasopressin, to determine ''masculine'' patterns of physiology and behavior, e.g., selective aggression [121]. Testosterone converted to estrogen can play a role in the central synthesis of vasopressin [30]. Neonatal exposure to vasopressin in the early postnatal period also can facilitate adult aggression, at least in prairie voles [103]. The relationship between parental behavior and androgens is not always negative [66]. For example, at least some aspects of paternal behavior in the monogamous California mouse require testosterone, which is in turn transformed by aromatization to estrogen [109]. Differential activity of enzymes (aromatase and reductase) responsible for the metabolism of testosterone may be an important source of species-typical and individual variance in both morphology and behavior.

Glucocorticoid hormones also have been implicated in development. Ontogenetic experiences, including levels of perinatal stress (including corticosterone) and varying amounts of parent–young interaction, can contribute to the development of species-typical patterns of social and parental behavior [15,37,61]. Another example of the consequences of perinatal exposure to stress hormones comes from work with prairie voles; in this species, corticosterone treatment during the perinatal period altered both social and reproductive behaviors. In female prairie voles, postnatal treatment with corticosterone was associated with an increased preference for unfamiliar partners versus siblings, lower levels of alloparenting and increased masculinization of sexual behavior (indexed by mounting behavior in females). A more stressful early life, including possibly the absence of the father, also inhibited alloparenting in female prairie voles from a population captured in Illinois [92–94]. In nature, a lack of preference familiar animals or unwillingness to engage in alloparenting behavior might be associated with less tendency to remain with the natal family, further undermining communal breeding and monogamous social systems [20].

However, even within prairie voles, intraspecific population differences exist in social behaviors, including juvenile alloparental behavior and other indices of communal breeding [20]. For example, animals from stock captured in a different habitat in eastern Kansas, although capable of forming pair bonds, are less likely to show the communal behaviors seen in animals of the same species that originated in Illinois. Thus, the traits of social monogamy show both intraspecific and interspecific variation. Understanding the ontogeny and phylogeny of such differences offers insight into the development consequences of hormones.

7.4. Morphological effects of OT

OT has been implicated in several remarkable cellular transformations. To the extent that any hormone can influence cellular growth or death, differentiation or contact with other cells, such a molecule holds the potential to remodel the nervous system. OT and steroid hormones, such as estrogen, which have regulatory influences over the oxytocinergic system, have been shown to influence many aspects of cellular function and differentiation [105].

Recent studies implicate OT in development and plasticity. For example, OT-producing neurons in the hypothalamus have unusual morphological features in comparison with other neurons. OT neurons in adult rats have been described as ''immature'' with exceptional capacity to change shape and form new synapses [107]. OT-producing cells are sensitive to OT itself; a form of autocrine feedback regulates the functions of OT-producing cells. Increases in OT from exogenous sources also can stimulate the production of OT from these cells.

OT has been implicated in cellular proliferation in vitro. In mouse P19 embryonic stem cells, OT has the capacity to induce cell aggregation and enhance the proliferation of cardiomyocytes [82]. These OT-induced aggregates of cells engaged in synchronous beating and over a matter of days express cellular markers considered specific to heart cells, including atrial natriuretic peptide (ANP); in turn, ANP also has been implicated in the regulation of cell growth. In addition, OT-treated stem cells began to express the OT receptor protein, which could in turn increase subsequent sensitivity of these such cells to OT. Antiproliferative effects of OT have been documented in breast cancer cells and other neuroplastoma and gliablastoma cell lines [21].

There is at present no published evidence for a direct test of the role of OT in stem cell development based on normal neural tissues or developmental models of neurogenesis. However, estrogen, which tends to enhance the actions of OT, has been implicated in neurogenesis in several mammalian models [105], including prairie voles [36,100]. Based upon the long-lasting effects that accompany early manipulations of OT described above and on the dramatic actions of OT in in vitro models, studies are in progress in my laboratory examining the hypothesis that developmental exposure to OT, with or without the aid of estrogen, might alter either neurogenesis and/or cell death.

8. OT and human development

Research on the developmental consequences of OT has been stimulated by concern for the possible long-term effects of manipulations of this peptide during early life. The concerns expressed in this paper are not new. For example, as stated by Boer [9], altered levels of OT and vasopressin in the perinatal mammal can induce ''lasting functional teratogenic effects'' acting through ''changed

levels of homeostatic set points of the functioning of peripheral and central OT and vasopressin systems, including the level and efficacy of their receptors…Do we therefore have to expect functional neuroteratological effects of enhanced vasopressin or OT in the perinatal period of human being? It is not to be excluded yet that OT-mediated induction of human labor can have such effects. The possible hazards of maternal OT infusion to the fetus have been discussed frequently…but none of these studies were designed to test long-term effects. However, according to us (i.e., Boer et al.), there might be an additional reason to substantiate that altered vasopressin and/or OT levels can play a teratogenic role or induce subtle lasting defects. At birth, the circulating levels of vasopressin and OT rise. So, either an elective caesarian (which prevents occurrence of these enhancements) or a complicated delivery (which elongates the period during which these high levels occur) may expose the brain and body of the child to a completely different vasopressin/OT signal…Hence, either during pregnancy or around birth, the conditions of exposure to vasopressin and OT may vary quite distinctly from child to child.''

It was assumed until about 20 years ago that OT was primarily a ''female reproductive hormone'' with major effects on peripheral smooth muscles in the uterus and breast. The possibility that OT had actions on other systems and in males was largely ignored. In addition, although it was long known that OT was made in the brain, including magnocellular areas of the hypothalamus (PVN and SON), it was initially believed that all of the hypothalamic OT was released into the general circulation at the posterior pituitary (neurohypophysis). It was further assumed that the blood–brain barrier prevented the direct return of neurohypophyseal OT to the CNS; thus, OT was viewed as a peripheral hormone. However, more recently, there has been a revolution in the understanding of the role of peptide hormones in brain function. It is now clear that peptides, including OT, are manufactured within the nervous system and released centrally to act upon a variety of CNS receptors [6,35,42].

Implicit in the routine use of OT in humans is the assumption that maternal OT does not cross the placental barrier in amounts that are sufficient to influence the developing fetus. Measurements of peptides in fetal rats and human umbilical blood provide some support for this assumption [85]. However, radioimmunoassays for OT are relatively insensitive, and more refined methods for analysis have only recently become available (Kramer et al., unpublished data). In addition, effects of peptide manipulations can be indirect, e.g., through the consequences of uterine contractions. Proof that the fetus is not influenced by maternal peptides requires functional and in vivo measures of the effects of OT. In addition, exogenous OT, OTAs or other peptides may have effects that differ from those of endogenous OT. Furthermore, the permeability of the maternal–fetal barrier could fluctuate during labor and birth, which is the period when medical manipulations of OT are most common. Postpartum OT treatments of the mother also are sometimes recommended on the assumption that these will reduce the chances of postpartum hemorrhage, providing another possible opportunity for the nursing infants to encounter elevations in OT.

As mentioned above, patterns of infant care and feeding also may be translated into peptidergic manipulations. For example, because human milk [62], but perhaps not heat-treated baby formulas, contains comparatively high levels of the OT molecule, the decision to bottle-feed an infant also may constitute a manipulation of the peptide history of a child. In addition, breast-fed infants may receive more physical contact, including skin-to-skin contact and oral stimulation, than bottle-fed infants, which may in turn release OT in the infant (or mother) [113].

8.1. Pitocin

OT treatment, in the synthetic form known as pitocin, is used routinely to hasten labor and delivery [34,108]. Because opioid-based analgesics can inhibit the endogenous release of OT [33,63,95,96] and thus uterine contractions, women who receive painkillers during labor often require OT to reinstate labor. OT-facilitated deliveries are on average shorter (about 2.5 h) with more intense contractions, and concomitant need for pain relief, when compared with births that do not involve the use of pitocin [108]. OT supplements are used in the United States in a high percent of births, especially in primiparous women. Hospitals that adhere to a procedure known as ''active management of labor'' may recommend exogenous OT for virtually every woman in labor. The use of OT has become so widespread that there is a tendency to assume that its effects are well known and benign, and increased use and even higher doses have been recommended, in some cases in an attempt to reduce the need for caesarian section [34,108].

In spite of the widespread use of pitocin, there are very few studies of the role of OT in development. At the level of behavior, the assumption that perinatal OT manipulations are without effect is largely untested, although the small, but growing, literature in animals suggests that this may be an invalid assumption [9,10,114].

The apparent or perceived need to augment natural amounts of OT during labor is based in part on the assumption that a subset of women do not produce an amount of OT that is sufficient for normal birth or that some women are unable to respond to the endogenous OT that is available through natural means. Proponents of ''natural'' childbirth have argued that obstetric practices interfere with the release of endogenous OT. Caesarean sections also affect the subsequent release in the mother of pulses of OT as well as maternal responses to the infant [79]. At present, in the United States, about 20% of infants are delivered by Caesarean sections. This percentage has risen from $\sim$5.5% in 1970 (National Center for Health Statis-

tics). In some hospitals serving high-risk populations, the caesarian rate approaches 50%. Women who are currently giving birth may be second- and third-generation products of the use of various obstetric and child rearing experiences that involve or influence OT. Whether there is an intergenerational consequence of early exposure to peptide manipulations remains to be determined.

8.2. OT antagonists

Prematurity is a major medical problem throughout the world. To quote one author, "It is the single most important cause of death among infants and a major cause of newborn and child morbidity. Thus, its prevention is an important public health goal" [69]. Therefore, many laboratories and pharmaceutical companies are in the process of testing OTAs for their capacity to inhibit labor and one compound (Atosiban, Ferring Pharmaceuticals) is approved for clinical use in Europe and is gaining wide acceptance [47]. In some cases, Atosiban may be used prophylactically in mothers at risk for premature labor. Methods for preventing premature labor remain a major medical concern. However, testing for OTAs has tended to focus on the delivery process and immediate effects on the newborn. Long-term consequences for behavior and physiology seem to have received little attention.

8.3. Breast-feeding

OT [62], along with many bioactive hormones and growth factors, also occurs naturally in human milk but probably not in infant formulas. In addition, the endogenous release of OT within the infant itself may be increased by suckling and tactile stimulation. For example, it has been shown in both young dogs and calves that nonnutritive suckling itself releases OT in the infant [113]. Babies that are bottle fed may have a different nursing experience (using less pressure) and under some child rearing conditions receive less tactile stimulation because they are held less. Thus, the decision to breast-feed or bottle-feed a child is a potential manipulation of the peptide experience of the newborn. The degree to which bioactive compounds in milk, or changes in OT or other peptides, produced by the infant itself that are secondary to suckling and/or maternal contact, affect physiology or behavior has only recently become the subject of serious investigation. Still there is evidence that such early hormonal manipulations may affect either the mother and/or the infant, having consequences ranging from brain growth [9,11] to later stress reactivity [46,90] to ovarian disorders [45]. Taken together, these and a variety of other data (reviewed in Refs. [13,15,16,113]) support the broader hypothesis that both mother and child may benefit from breast-feeding. Whether OT is involved directly or indirectly in these benefits is at present unknown.

The capacity of OT to pass from the mother's to the child's digestive system and to remain functionally intact or to maintain functional fragments has not been studied. However, research in rats has shown that the presence or absence in maternal milk of the related hormone, prolactin, which is a larger molecule, can permanently modify endocrine function in the offspring [45]. Following treatments that removed most of the endogenous prolactin from milk on postnatal days 2–5, female rats developed various indices of reproductive dysfunction including, as adults, hyperprolactinemia and nonovulatory, polycystic ovaries. Replacement of prolactin during development restored normal function. These findings document the capacity of hormones in milk to have long-term physiological consequences. OT and prolactin have a variety of physiological interactions and may act as releasing factors for each other under natural conditions synergizing to permit normal lactation [95]. Thus, the presence or absence of prolactin might have indirect effects on OT and vice versa.

The presence of OT in breast milk and the effects of other aspects of child rearing on OT may be manipulations of peptidergic systems in the developing organism. Furthermore, because it is now known that tactile stimulation (along with other sensory modalities) can induce the release of OT [113], various aspects of maternal–child interactions beyond the actual nursing, including the amount of time spent holding the infant, hold the potential to influence the OT system.

8.4. Other clinical implications

OT interacts at several levels with the endogenous opioids. Opioids regulate the release patterns of OT [33,63]. There is evidence that the addictive properties of various drugs of abuse, including opiates and cocaine, may be influenced by (or conversely affect) endogenous OT [54,60]. Such interactions might help to explain why the social environment (e.g., the presence or absence of social support, which could influence OT) is of such importance in regulating the development and recovery from addictions of all sorts.

OT has been implicated in autism [50]. In certain forms of autism, OT levels are low [73]. It has also been recently reported that not only are OT levels depressed but also that levels of the OT precursor, termed OT-X, are higher in some autistic children than in controls [44]. OT-X is more prevalent in fetal life, and enzymatic processing to OT normally occurs more completely as the fetus matures [75]. Whether early experiences or manipulations of peptides around the time of birth could affect these enzymes remains to be studied.

9. Summary

Positive relationships and social support have repeatedly been related to health [98,111]. The link between OT and

positive social behaviors also is strong, and it is possible that the protective effects of social bonds may be mediated in part through the physiological actions of OT [15,46,59].

Social behavior and social relationships are inherently variable, with patterns that differ among species and individuals. Endogenous OT or its receptors and related peptides such as vasopressin may affect and be affected by various social experiences, especially during early life. The neural systems that incorporate OT also are plastic. OT, acting on its own receptors and through effects on other systems including the HPA axis and the autonomic nervous system, has a powerful capacity to alter physiology and behavior. There is in vitro evidence that OT can affect cell proliferation or cell death [21,82]. Research in animals suggests that the OT system and its targets are sensitive to ''tuning'' in early development, with lifelong consequences.

Medical and pharmacological manipulations, e.g., methods of childbirth, caesarian section and use of OT (pitocin) and OTA (e.g., Atosiban), also have the potential to influence these peptidergic systems. Such actions might be mediated through the mother or through direct effects on infants. Understanding the mechanisms and long-term effects of such treatments offers a medical challenge as well as possible insight into the developmental consequences of OT.

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