4

The organizational effects of oxytocin and vasopressin

Behavioral implications

Bruce S. Cushing

4.1 Introduction

The critical role of the neuropeptides oxytocin (OT) and arginine vasopressin (AVP) in regulating social and socio-sexual behavior, from social recognition to mating to pair-bond formation to parental care, has been a major research focus for the past 30 years. The importance and wealth of information that has resulted makes for great reading, as evidenced by most of Part II, Chapters 5–16, being committed to covering this "modest" subject. Not to give the punch line away, but Part II reveals at least two critical things, first that the vast majority of research has focused on adults and secondly that while both OT and AVP may regulate many of the same behaviors in males and females the effects are often sexually dimorphic with OT playing a greater role in females and AVP in males (Cushing et al., 2001; Insel and Young, 2001). In Chapter 1 (Caldwell et al., this book) another critical aspect of the effects of OT and AVP in adults is discussed, which is that many of the behavioral effects, especially the regulation of social and socio-sexual behavior, are steroid dependent. Increasing steroid levels either enhance or are necessary for the behavioral effects of OT (estrogen) and AVP (estrogen and dihydrotestosterone) (see Caldwell et al., Chapter 1). This relationship is perhaps not surprising given that many of the behavioral and even physiological responses regulated by OT and AVP are associated either directly or indirectly with reproductive effort, that is, investigation of novel individuals, social recognition, social memory (for a review see Chapter 13), mating (Chapter 14), pair-bond formation (adult and maternal infant), parturition, maternal (Chapters 8–10) and paternal behavior (Chapter 8), in which sex steroids typically play a major role.

In contrast to this large body of research there is an emerging interest in the effects of these neuropeptides during development, both pre- and postnatal, on the ultimate expression of physiological and social behavior. While the hypothesis that AVP and OT may have an organizational effect within the brain producing long-term/permanent changes was proposed in the mid to late 1980s (AVP: Boer, 1985; OT: Noonan et al., 1989) the study of this phenomenon did not spark the same level of interest or funding as neuropeptide regulation in adults and languished compared to examination of post-pubertal affects. However, the end of the twentieth and the beginning of the twenty-first century have seen a rebirth of interest in developmental effects producing increasing primary research and a flourish of review papers examining the role of neuropeptides during early critical periods and their potential relevance to social deficit disorders (Carter et al., 2009). Although I may be a bit biased, these studies and papers have revealed potentially critical findings, which have lead to rethinking the role of neuropeptides. Major findings

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include: (1) demonstration of an "organizational" effect of neuropeptides, (2) redefinition of the relationship between neuropeptides and steroids, and (3) demonstration of the critical importance of the early environment, including, the early social environment, in epigenetic/non-genomic regulation of not only the expression of adult behavior, but also in transgenerational transmission, and (4) stimulation of research on neuropeptides as a mechanism of, and possible treatment for, social deficit disorders, such as autism, schizophrenia, depression, and PTSD. Therefore, the goal of this chapter is to summarize the essential findings of this research discussing long-term behavioral and sexually dimorphic effects, the underlying mechanisms of effects/actions, the non-genomic effects, and the future direction of research of OT and AVP during development.

The understanding and implications of developmental effects vary between OT and AVP for several reasons: (1) OT has only one known, highly conserved, receptor. In contrast AVP has multiple receptors, V1 and V2, which have additional subtypes, that is, V1A and V1B. While the distribution of these receptors varies, V2 being found primarily in the periphery, all types are found in the brain and both of the V1 subtypes regulate behavior. This means that sorting out the effects and actions of vasopressin requires receptor-specific antagonists and agonists. (2) In knockout models there could be compensatory receptor expression for vasopressin receptor knockout mice, but not OT receptor knockout mice. (3) It is difficult to directly manipulate hormones within the CNS during development. In adults, OT, AVP, and their antagonists can be delivered directly via injection into ventricles or to site-specific regions in the brain. Although this has been accomplished in a few acute studies (Chen et al., 1988; Boer et al., 1994; Stribley and Carter, 1999) direct manipulation within the CNS is much more difficult, and therefore peripheral injections have been typically used for developmental studies. With a reduced blood–brain barrier in fetuses and neonates (Vorbrodt, 1993) this is an effective method for manipulating CNS affects. However, the peripheral administration of AVP has the potential to have a more profound effect than OT. Although both AVP and OT can affect a number of systems and responses peripheral manipulations of AVP has the potential to cause much greater adverse effects in both the adult and during development. Acting via the V2 receptor AVP plays a major role in water balance and therefore circulating levels can and do have a deleterious effect on an individual's physiology. In contrast, OT tends to affect smooth muscle contraction and many of the effects are related to adult behavioral responses.

4.1.1 Historical perspective

The vasopressin-deficient Brattleboro rat provided early indication of the potential role of vasopressin during development. Neonatal peripheral treatment with vasopressin permanently alters some systemic responses, including cardiac, and from this Boer (1985) hypothesized that AVP might also have an organizational effect within the central nervous system. The finding that during embryonic development treatment of pregnant females with vasopressin altered the development of the brain (Boer et al., 1988) supported the concept of an organizational effect. The development of the brain was associated with vasopressin acting via the V1 type receptor, as brain development in Wistar rats was only affected by a V1 antagonist, but not a V2 antagonist (Snijdewint and Boer, 1988). Neonatal treatment of pups resulted in increased emotionality in females and increased ambulatory behavior in males in an open field test (Boer et al., 1994). Results from this study provided some of the first empirical evidence of long-term behavioral changes associated with vasopressin during postnatal development, and support for an organizational effect. It also demonstrated that neonatal effects are sexually dimorphic. This study also tested the effect of repeated treatment with OT and found that as with a single injection of OT (Noonan et al., 1989, see below) neonatal treatment with OT did not affect behavior in an open field. These results indicated that neonatal effects were sexually

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dimorphic and that vasopressin and OT regulate different behaviors.

Noonan et al. (1989) proposed that OT could have long-term effects on behavior through organizational effects within the brain and tested this by treating rats with a single dose of OT on postnatal day three. The results supported the hypothesis, with neonatal OT increasing novelty induced grooming in both males and females. In 1989, Shaprio and Insel described the ontogeny of OT receptors in the rat and demonstrated that during postnatal development the expression of OT was transitory in many regions of the brain. Based upon this transitory nature they hypothesized a limited time period for organizational effects that predicted a significant difference between responses to OT during the neonatal period and adulthood. The ontogeny and topography of vasopressinergic and oxytocinergic neurons using mRNA was described in the rat in 1990 (Bloch et al., 1990), followed shortly thereafter by a description of ontogeny of vasopressin receptor expression in the fetal and infant rat brain (Tribollett et al., 1991).

While these studies demonstrated a potential organizational effect of OT and AVP they did not provide evidence as to the system or systems being affected. Boer had suggested that vasopressin could be altering responses by organizing the central vasopressinergic system. This hypothesis was based in part upon the finding that neonatal treatment has long-term effects on peripheral receptor expression in the heart and kidney. Brattleboro rats, which displayed vasopressin deficiency, also displayed higher than normal OT levels throughout life and offered the possibility that one neuropeptide might affect the sister neuropeptide (Boer et al., 1988). But it would be almost a decade before it would be shown that OT and AVP had major effects on the long-term expression of social behavior, and we began to demonstrate the systems being impacted by these neuropeptides during development.

4.2 Behavioral effects

The goal of this to provide an understanding of the impact that OT or AVP may have on the "ultimate" expression of behavior not to elucidate nor detail all of the behavioral effects associated with these neuropeptides during development. The behavioral effects of OT and AVP can be divided into two categories, those that are relevant to the neonatal period versus long-term effects. While it may be in part due to the nature of the research questions that were asked, the majority of studies indicate that the behavioral effects associated with OT and AVP influence or regulate behaviors that are relevant to adult social interactions rather than those associated with the developmental period. To a large extent these findings provide the support for and are the basis of the hypothesis of an organizational effect of AVP and OT. Age appropriate effects for OT include altered ultrasound production in response to maternal isolation in rats (Insel and Winslow, 1991) and prairie voles (Kramer et al., 2003). In adults OT has been shown to be anxiolytic, reducing responses to stress, (McCarthy et al., 1997) and these findings suggest that it may play the same role during the neonatal period. In fetal rats intrathecal injections of AVP increased fetal activity, including mouthing, licking, wiping, and intracisternal injection of V1 antagonists reduced wiping and oral grasping of an artificial nipple (Valinskaya et al., 1994). In contrast, intrahemispheric injections of a V1 antagonist increased wiping and grasping of an artificial nipple. AVP deficient rat pups displayed a different group dynamic, reduced huddling and proximity to other pups, which may be involved in social interaction (Schank, 2009), but could ultimately be involved in thermoregulation and water balance. Central administration of vasopressin in rat pups decreased ultrasonic vocalizations and locomotor acitivty (Winslow and Insel, 1995).

The majority of studies have examined the subsequent effects of neonatal treatment in adults. Neonatal manipulations have subsequent long-term effects on many of the same behavioral and physiological responses that these neuropeptides regulate in adults, indicating that during the neonatal period they function to establish adult behavior. Given the nature of the critical role of OT in regulating social behavior much of the research on neonatal effects of these peptides has been

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conducted using the highly social prairie vole. In prairie voles neonatal manipulation of OT affects the subsequent expression of a number of socio-sexual behaviors in adults. These included, but are not limited to, aggression (Bales and Carter, 2003a), alloparental behavior (Bales et al., 2004b), formation of partner preferences (Bales and Carter, 2003b), mating (Cushing et al., 2005), and reproductive success (Bales et al., 2004a; Cushing et al., 2005). In the Mandarin vole (*Microtus mandarinus*) neonatal treatment increased the probability that females, but not males, would form a partner preference, and increased reproductive activity in males (Jia et al., 2008). There are only a few studies examining the effects of neonatal AVP on the expression of adult behavior. This may be due in part to the constraints of treating with AVP during the neonatal period (see historical perspective). Neonatal manipulation of vasopressin, and selective inhibition of V1a receptor have been shown to have long-term effects on aggression (Stribley and Carter, 1999), mating (Meyerson et al., 1988), and open field behavior (Boer et al., 1993). While the effects of OT and AVP are typically sexually dimorphic, in adults, with AVP playing a greater role in males and OT in females (Cushing et al., 2001; Insel and Young, 2001), in neonatal treatment the degree of sexual dimorphism depends upon the behavior being studied. In some cases one may regulate the behavior in males and the other in females. For example in prairie voles neonatal treatment with AVP increased aggression in adult males but not females (Stribley and Carter, 1999), while neonatal OT increased aggression in females but not males (Bales and Carter, 2003a). In other cases they have similar effects, for example, in the prairie vole early treatment with OT enhanced the formation of pair-bonds and partner-preference in both male (Bales and Carter, 2003b) and female (Bales et al., 2007). However, the response may also be species specific as in Mandarin voles neonatal treatment, without mating, only enhanced partner preference in females (Jia et al., 2008). Finally for some behaviors the effect of OT may be limited to males. Male prairie voles typically display higher levels of spontaneous alloparental behavior than females (Roberts et al., 1998). Use of selective OT antagonist during neonatal development significantly reduced male, but not female, (Bales et al., 2004a), indicating that inhibition of endogenous OT has sexually dimorphic effects.

4.3 Early social environment

The early postnatal environment, especially in mammals, is a period of intense social interaction, maternal, sibling, and in some species paternal or with other relatives. These early experiences can and do influence the subsequent expression of social behavior. This critical period is a time of bidirectional affects, where the early environment may effect the development and sensitivity to hormones and well as the hormones affecting the neonatal social interactions. Early social experience affects the oxytocinergic and/or vasopressinergic system. In rats, the early social environment altered the subsequent expression of adult social behavior in both males and females (Francis et al., 1999). This was associated with site-specific increases in OTR in females and V1a in males (Francis et al., 2002). Maternal care may be directly affecting endogenous OT levels as contacts between mother and infants are known to increase OT production (Uvnäs-Moberg et al., 1998). Conversely, maternal separation reduced the subsequent expression of socio-sexual behavior. In rats maternal separation resulted in a decrease in OT neurons in the paraventricular nucleus of the hypothalamus (PVN) of females and AVP in males (Todeschin et al., 2009), which was associated with increased aggression and reduced social investigation by males. In another study long-term maternal separation produced a decrease in OT immunoreactivity in the hypothalamus and amygdala of three-week-old male rat pups (Oreland et al., 2010). Maternal separation also affects AVP and OT receptors in males (Lukas et al., 2010).

The effect of the early social environment may not only be expressed in the adult, but also has been shown to be transgenerational in both rats (for review see Champagne, 2008) and prairie voles (Stone and Bales, 2010), with implications for cross-generational OT transmission in humans

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(Feldman et al., 2010). The transgenerational and long-term effects have been associated with epigenetic effects on estrogen receptor alpha (ERα) expression and the interplay of estrogen and OT and AVP (for review, see Champagne, 2008; Shepard et al., 2009). These reviews bring together a significant body of literature on early environmental, genetic, and epigenetic effects with an emphasis on estrogen and ERα. In turn, they argue very eloquently that, at least some of the behavioral and physiological changes may then occur via the oxytocinergic or vasopressinergic systems because of the steroid-dependent nature of OT and AVP. They discuss possible mechanisms including corticotrophin releasing hormone (CRH) and other regulatory hormones. I would also suggest the intriguing hypothesis that it is the effects on OT and AVP of the early social experiences that could be involved in the epigenetic regulation of ERα. This might be the case as OT has been shown to have an organizational effect on ERα (see Section 4.4.1, Yamamoto et al., 2004; Kramer et al., 2007; Perry et al., 2009). This also makes logical sense from the standpoint that contact and social interaction can have a fairly rapid effect on OT release/production, while gonadal steroid responses are generally much slower and very limited in females with inactive ovaries.

4.4 Organizational effects

The long-term behavioral effects of manipulation of endogenous or exogenous OT during the neonatal period provide strong support for the hypothesis that OT has an organizational effect on the CNS (Noonan et al., 1989; Shapiro and Insel, 1989; Yoshimura et al., 1996). The mechanism or mechanisms of OT actions or the systems that are being affected are not clear and are a work in progress. One approach to investigating long-term organizational effects is to ask what response is being affected and to then determine if the underlying mechanisms associated with these behaviors or physiological responses have been affected. While the long-term effects of neonatal OT cover a wide variety of responses they can be classified into two major categories : (1) Socio-sexual behavior, including aggression, pair-bond formation, mating, parental care, etc., and (2) Stress, such as social isolation, novel encounters, cardiac response, and corticosterone levels. Not surprisingly these are many of the same behaviors and responses that have been shown to be regulated/influenced in adults (see Chapters 5–16).

4.4.1 OT and estrogen

In adults, especially females, the behavioral effects of OT are estrogen dependent (Choleris et al., 2003). However, in mammals the ovaries are inactive during the fetal and neonatal period and therefore little or no estrogen is produced. This means that the dependence on steroids is highly unlikely during the neonatal period. In contrast, during the neonatal period many of OT's long-term effects are on estrogen-dependent behaviors, suggesting the hypothesis that during the neonatal period OT could be organizing subsequent response to estrogen. In rats, OT-treated females weighed significantly more after puberty and the increase was associated with fat depositions regulated by estradiol (Uvnäs-Moberg et al., 1998) and placental and fetal growth during pregnancy as adults (Sohlström et al., 2002). Neonatal OT influences the onset of first estrus and vaginal opening in female rats (Withuhn et al., 2003; Parent et al., 2008). In female prairie voles a single neonatal treatment on the day of birth affected sexual receptivity and the probability of successfully producing a litter (Cushing et al., 2005). Female prairie voles undergo induced estrus with exposure to males, increasing estrogen levels and stimulating mating. Mating is also associated with increased female/female aggression and neonatal treatment with OT simulates the effects of estrogen by an increase in aggression following exposure to a male in both prairie voles (Bales and Carter, 2003a) and Mandarin voles (Jia et al., 2008b).

Studies in undifferentiated cancer cell lines and the developing ovary suggested that the developmental stage may play a role in the ability of OT to regulate the response/sensitivity to

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estrogen. In MCF7 breast cancer cells treatment with OT inhibited the ability of estadiol to stimulate mitosis (Cassoni et al., 1997). OT had a direct effect on several aspects of ERα expression including production of ERα mRNA, binding affinity, and transcriptional activity (Cassoni et al., 2002). Cancer cells are less differentiated than other cells, and "capable" of undergoing the equivalent of organizational effects. In prairie voles, OT treatment increased sensitivity to estradiol, lowering the threshold dose required to trigger estrus in sexually naïve females, but had no effect in sexually experienced females (Cushing and Carter, 1999). Unlike most female mammals, the completion of sexual development in prairie voles requires chemical and social cues from males (Carter et al., 1987). Regardless of chronological age, females are not sexually mature so that a sexually experience female and a sexually naïve female represent significantly different developmental stages. The differential response to OT suggests that the stage of development is associated with the response to OT manipulation and that puberty may alter the relationship between OT and estrogen, with the behavioral effects becoming steroid dependent, as observed in rats (Ivell and Walther, 2002). Outside of the central nervous system (CNS) there is evidence that during the neonatal period OT can affect development. In rats, neonatal OT affected apoptosis in the developing ovary (Marzona et al., 2003). Since the ovary is the primary site of estrogen production changes in the cellular composition of the ovary during development could alter the subsequent production of estrogen and/or ovarian sensitivity to LH, FSH, and estrogen. Finally, in rats maternal behavior altered central OT (Francis et al., 2002) and expression of ERα in females (Champagne et al., 2003) and vasopressin receptors in males (Francis et al., 2002).

The direct effect of endogenous and exogenous OT on the expression of estrogen receptors in the neonatal period was determined in two rodent species with distinctly different social systems, the highly social prairie vole and Sprague Dawley rats. In two studies, male and female prairie voles received a single injection of OT, a selective OT antagonist (OTA), or saline control on the day of birth (P1). The expression of ERα was then examined on P1, P8, P21, and in adults. While not discussed in detail here it should be noted that the expression of ERα is sexually dimorphic in adult prairie voles with females expressing significantly more ERα than males (Hnatczuk et al., 1994; Cushing et al., 2004) and this difference is apparent by P21 (Yamamoto et al., 2006). The results support the hypothesis that OT has an organizational effect of ERα expression and also indicate that the effects of OT are sexually dimorphic. In females, by P21 site- and treatment-specific effects were apparent with OT producing a significant increase in ERα in the ventromedial hypothalamus (VMH) (figures 4.1 and 4.2), while OTA treatment produced a significant decrease in ERα in the medial preoptic area (figures 4.1 and 4.2) (Yamamoto et al., 2006). In contrast, effects in males were not observed until P60, with OTA increasing the expression of ERα in the bed nucleus of the stria terminalis (BST) (Kramer et al., 2007). The prairie vole is a valuable model

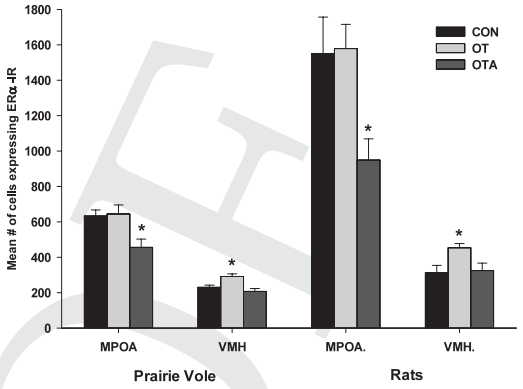

Figure 4.1 Effects of neonatal oxytocin (OT) manipulation on the number of cells expressing ERα immunoreactivity in the MPOA and VMH in female prairie voles and female rats. In both prairie voles and rats inhibiting the effects of endogenous OT with and OT antagonist (OTA) significantly decreased the number of cells expressing ERα in the MPOA, while treatment with exogenous oxytocin (OT) significantly increased the number of cells expressing ERα in the VMH compared with controls (CON). * = significantly different from control (vehicle treated) females ($p < 0.05$). Prairie vole data adapted from Yamamoto et al. 2006 and rat from Perry et al. 2009.

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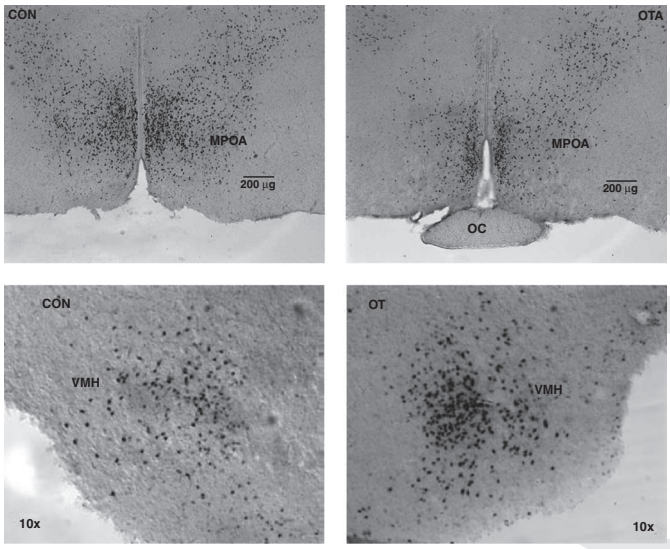

Figure 4.2 Photomicrographs of ERα immunoreactivity in female prairie voles on postnatal day 21 in response to manipulation of OT on the day of birth. 4× (40× magnification) represent ERα immunoreactivity in the MPOA in Control (CON) and oxytocin antagonist (OTA) treated females. 10× (100× magnification) shows the representative increase in ERα in the VMH resulting from OT treatment. Adapted from Yamamoto et al. 2006.

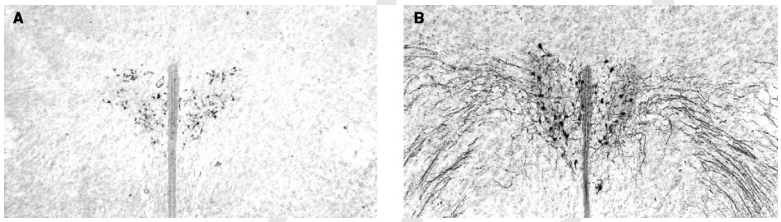

Figure 4.3 Photomicrographs of ERα immunoreactivity in female rats in response to manipulation of OT on postnatal days 1–7. ERα immunoreactivity in the MPOA in Control (CON) and oxytocin antagonist (OTA) treated females. 10× (100× magnification) showing the representative increase in ERα in the VMH associated with OT treatment. Adapted from Perry et al. 2009.

for studying human relevant social behavior in part because it is socially monogamous, monogamy is a rare trait found in only 3 to 5% of mammalian species (Kleiman, 1977). To determine if the organizational effects of OT on ERα is a general phenomenon or specific to highly social species the effects of neonatal OT manipulation were investigated in female rats. The effects of neonatal OT manipulation in female rats was the same as that observed in female prairie voles with OT increasing ERα expression in the VMH (figures 4.1 and 4.3) and OTA decreasing ERα expression in the (MPOA) (figures 4.1 and 4.3) (Perry et al., 2009). These results clearly demonstrated that the organizational effects of OT are not species specific and while the effects on the ultimate expression of behavior may differ, the effects on the underlying mechanisms are the same. Further results indicate there may be a dose-dependent effect of OT during the neonatal period as female prairie voles treated with a lower dose of OT on P1 also displayed an increase in ERα in the lateral septum (LS) and

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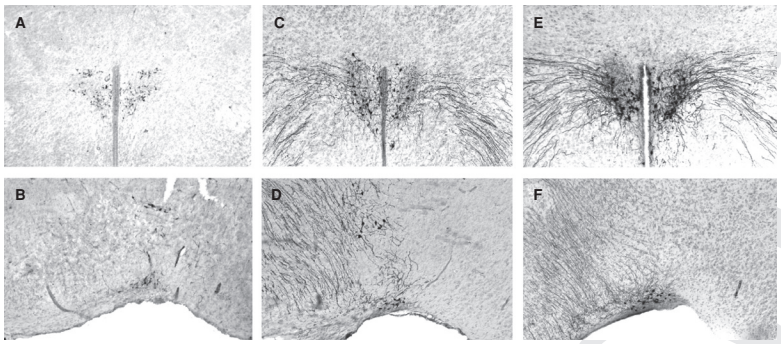

Figure 4.4 Photomicrographs of oxytocin expression in the paraventricular nucleus of the hypothalamus (A, C, E) and the supraoptic nucleus (B, D, F) on postnatal day 1 (A + B), day 8 (C, D), and day 21 (E, F) in female prairie voles. Adapted from Yamamoto et al. 2004.

central amygdala in addition to the VMH (Kramer et al., 2007). The fact that OT has an organizational effect on ERα could be highly significant as it suggests the possibility that non-genomic events, such as early social experiences that influence neonatal OT expression, can have a major impact on the ultimate expression of social behavior and influence social interactions.

Although the mechanism by which OT alters the expression/organizes ERα is unknown findings from studies of non-neural tissue provide potential options. In breast cancer cells, which are less differentiated than adult cells, OT treatment directly affected the expression of ERα. Effects of OT included altering ERα mRNA production, binding affinity, and transcriptional activity (Cassoni et al., 2002), while in the neonatal ovary OT affected the rate of apoptosis (Marzona et al., 2003). *In vivo* studies in prairie voles support the hypothesis that neonatal OT can directly affect the expression of ERα, as within 2 h of OT treatment on P1 the expression of ERα mRNA was altered. OT treatment significantly increased ERα mRNA in the hypothalamus and hippocampus, but not the cortex, while OTA decreased ERα mRNA in the hippocampus (figure 4.4, Pournajafi-Nazarloo et al., 2007a). These effects were mRNA specific as ERβ mRNA expression was unaffected. The direction of the effect is consistent with the effect of neonatal manipulation on ERα-IR (Yamamoto et al., 2006; Perry et al., 2009) with OT increasing and OTA decreasing ERα. The effect of OT on ERα mRNA was not limited to the brain as OT treatment also altered the expression of ERα mRNA in the heart of both prairie voles (Pournajafi-Nazarloo et al., 2007b) and rats (Pournajafi-Nazarloo et al., 2007c). The effect in the heart suggests two things. First, the organizational effect of OT on ERα could in part be responsible for changes in cardiovascular response/performance reported in response to prepubertal OT treatment (Uvnäs-Moberg et al., 1998; Holst et al., 2002; Grippo et al., 2007). Second, the organizational effects of OT are not limited to the CNS. If neurons responded to OT like breast cancer cells then OT could be altering affinity and transcription of ER, which could account in part for longer-term effects and could contribute to the ultimate expression of ERα and sensitivity to estradiol.

Our lab tested the hypothesis that neonatal OT may be altering the number of cells that express ERα through regulating apoptosis. Neonatal treatment of prairie voles did not support this hypothesis as there was no evidence that OT altered apoptosis or apoptosis in ERα expressing neurons (unpublished data). In females treated with OT or OTA on P1 the number of apoptotic cells, as indicated by staining for TUNEL, was low throughout the limbic system, on P8 and P14, and there was no colocalization with ERα-expressing neurons. This suggests that the effect of OT differs between ovarian tissue and CNS neurons, and further implies that in neurons OT is acting by directly affecting ERα expression.

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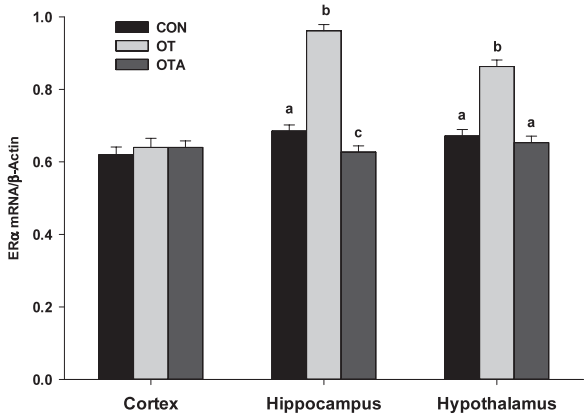

Figure 4.5 Effects of manipulation of OT on day 1 in female prairie voles on ERα mRNA 2 hrs after treatment. Treatment with oxytocin (OT) increased ERα mRNA relative to control (CON) and females treated with an OT antagonist (OTA) in the hippocampus and hypothalamus, but not the cortex, while OTA treatment reduced ERα mRNA in the hippocampus. Bars with different letters are significantly different from one another ($p < 0.05$). Adapted from Pournajafi-Nazarloo et al. 2007a.

Responses to OT and AVP are sexually dimorphic and have been linked to sexually dimorphic expression of several social deficit disorders, including depression, schizophrenia, and autism (Heinrichs et al., 2009). While I may admittedly be one of the few that find this perplexing, there is no indication that either during development or in adults that the oxytocinergic system is sexually dimorphic, so how then can OT be associated with sexually dimorphic responses? The organization of ERα could explain in large part the sexually dimorphic effect. During development the male gonad actively produces testosterone, which is converted intracellularly to estradiol by aromatase, while in the female there is little or no estrogen production until the ovaries become active at the onset of puberty. Therefore, changes in ERα expression would have differential effects in males and females. Additionally, organization of ERα would have a significant impact in adult females when the oxytocinerigc system is estrogen dependent (see Chapters 1 and 9).

4.4.2 Other organizational effects

Organizational effects on ER may explain many of the long-term changes in behavior. However, there are a number of reasons why it is unlikely that changes in response to steroids are sufficient to explain all of the effects of OT. First, during pre- and postnatal development the ovaries are inactive. This means that neonatal females would show little or no response to changes in ERα because there is no steroid to bind to the receptors. As such, changes in ERα are unlikely to explain changes in behavior that occur during the neonatal period. Second, in adults as well as neonates, behavioral and physiological responses to stressors are frequently rapid, and these types of responses are not typically associated with changes in nuclear receptors. Activation of nuclear receptors, ERα, involves transcriptional and translational activity, which are not considered rapid responses. Thirdly, many of the affected behaviors are also influenced or regulated by other mechanisms, including OT and AVP (for a review, see Cushing and Kramer, 2005), which at the very least suggests there could be changes in these or other underlying mechanisms.

Although the evidence is still limited there are studies that indicate OT may affect/organize other systems. OT may affect both neonatal responses and have long-term effects through the oxytocinergic system. In both prairie voles (Yamamoto et al., 2004) (figure 4.5) and rats (Perry et al., 2009) neonatal OT and OTA treatment increased the number of cells expressing OT in the PVN. The long-term effect on OT production is species specific. In female rats the increase in the OT positive cells in the PVN continued into adulthood (Perry et al., 2009), while adult female prairie voles no longer displayed differential production of oxytocinergic neurons (Kramer et al., 2007). The variation could be the result of a differential species response or the fact that in the vole study females only received a single treatment on P1 while rats were treated from P1–P6. It seems most likely that the difference is species specific, as a single treatment on P1 in female rats produced long-term increased OT production

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in the posterior pituitary of adults (Young et al., 2005).

Only one study has examined the potential effect of neonatal OT on CNS neuropeptide receptor expression. In this study, P1 OT manipulation did not alter the expression of OTR but did affect V1a receptor binding in adult prairie voles (Bales et al., 2007). The effects were site specific and sexually dimorphic. In females, P1 treatment reduced V1a binding, with OT decreasing binding in the MPOA, BST, LS, medial dorsal thalamic nucleus, and the Cingulate Cortex, while OTA treatment produced a reduction in binding in the BST and Cingulate Cortex. In males, OT treatment increased V1a binding in the Cingulate Cortex, while OTA treatment had the opposite effect resulting in a decrease in V1a binding in the MPOA, BST, and LS. Most of these regions regulate social behavior and the medial dorsal thalamic nucleus is one of the regions that displayed increased neuronal activity in response to neonatal OT treatment (Cushing et al., 2003). This means that neonatal effects of OT could alter behavioral response through an organizational effect on the receptor of its "sister" nonapeptide. This could explain sexually dimorphic effects.

Interestingly, this same study did not find an organizational effect of OT on OTR expression in either males or females. This, however, does not rule out the possibility that OT does affect the expression of OTR. In the heart, OT manipulation on P1 resulted in a change in OTR, as measured by real-time PCR, in both P21 rats (Pournajafi-Nazarloo et al., 2007c) and P21 prairie voles (Pournajafi-Nazarloo et al., 2007b). However, these studies did not examine the effects in adult hearts, so it is unknown if these changes persisted. In the prairie voles, neonatal effects on OT production in the PVN were observed on P21 but not in adult females, indicating that, at least in prairie voles, while the effects are significant they were not permanent. If the same thing happened in the brain then OTR expression would need to be looked for at an earlier age. In contrast, in rats the effects on PVN OT neurons was long term and still apparent in adult females. The difference between voles and rats suggests that it would be valuable to determine if there was also a long-term effect on OTR in female rats. If this were the case it would support the concept of OT playing a significant role in the expression of species-specific social behavior. The differential response also might be indicative of the change in the relationship between OT and estrogen that occurs in most adult females, but not prairie voles.

The study of the organizational effects of OT and AVP is a wide open field with tremendous potential and implications for understanding both basic regulatory mechanisms and possible prevention and treatment of social deficit disorders. The surface has barely been scratched in terms of the effects on ER, let alone OT and AVP. There are also indications that OT may have other major long-term organizational effects. In the heart, neonatal treatment affected endothelial nitric oxide synthase expression (Pournajafi-Nazarloo et al., 2007c) and we have recently demonstrated that neonatal OT has site-specific organizational effects on the serotonergic system (Eaton et al., 2011), which fits with the anxiolytic effects of OT and may explain, at least in part, the ability of OT treatment to prevent the onset of depression (Grippo et al., 2009; Heinrichs et al., 2009).

4.5 Ontological effects

Critical periods, when hormones, neurohormones, neurotransmitters, and other compounds have windows of time in which they can act to organize the brain, may occur throughout the life of an organism, but the developmental period, both embryonic and neonatal, typically represents a period when many changes are occurring. Therefore, the neonatal effects of OT and AVP are dependent upon the ontogeny of the oxytocinergic and vasopressinergic systems. While there are significant differences between the ontogeny of these systems, one thing that stands out is that there is little or no sexual dimorphism in these systems. In eutherian mammals the vasopressingeric system develops during

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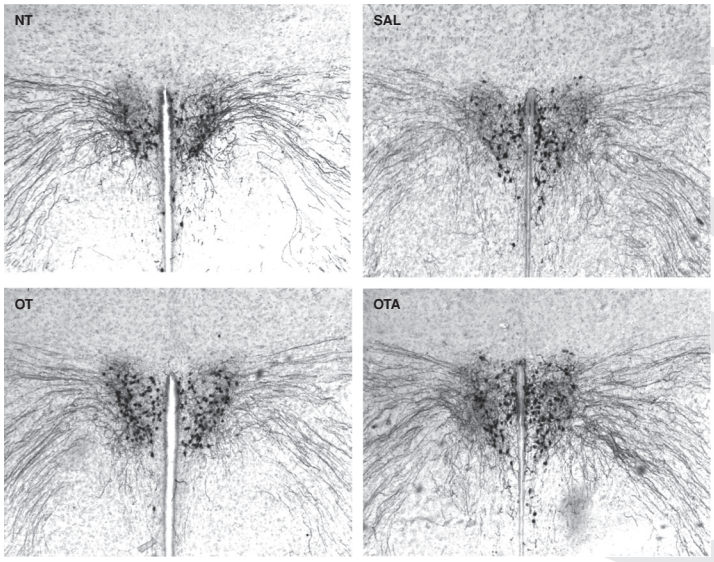

Figure 4.6 Photomicrograph of oxytocin expression in the paraventricular nucleus of the hypothalamus on day 21 in response to neonatal manipulation of oxytocin (OT) on the day of birth. Treatment with OT and an oxytocin antagonist (OTA) both resulted in an increase in the number of cells expressing OT compared to untreated (NT) and saline, vehicle, (SAL) treated females. Adapted from: Yamamoto et al. 2004.

the embryonic period (E), while the oxytocingeric system develops postnatally (P).

4.5.1 Oxytocinergic system

In mammals, central OT production either begins just prior to birth or shortly after. In mice, OT mRNA has been detected several days before birth on E18.5 (Jing et al., 1998), but peptide production is not observed until the early postnatal period, P1, in prairie voles (Yamamoto et al., 2004), and P4 or P7 in rats (Choy and Watkins, 1979; Altstein and Gainer, 1988). While OT was present in cell bodies of the PVN in prairie voles on P1 it was not until P8 that oxytocingeric fibers were observed emanating from the cell bodies (figure 4.6) (Yamamoto et al., 2004), suggesting that there may be a delay between production and release.

The ontogeny of OTR may explain much of the organizational effects of OT during the neonatal period and either the loss of effects or the dramatic changes in response observed in adults. Neonatal OTR expression is considered to be transitory with many regions expressing OTR only during early, preweaning, postnatal development. The trajectory of OTR expression also varies so that the period of maximum OTR expression differs by region across postnatal age (Choy and Watson, 1979; Shaprio and Insel, 1989; Jing et al., 1998; Chen et al., 2000). Taken together this means that first, there is definitely a neonatal critical period and second, the effects of OT should vary across the neonatal period, beginning on P1. Therefore, a single treatment on P1 would be predicted to have a different effect from one on P8, because different regions would be responsive. Paradoxically, a single treatment on P1 might not differ from repeated daily treatments starting on P1. A single treatment with OT during the neonatal period increased the number of OT neurons in the PVN (Yamamoto et al., 2004) and the production of OT in the posterior pituitary (Young et al., 2005). The results of early treatment indicate an initial positive feedback system with increased OT increasing endogenous OT

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production, which could then interact with the changing pattern of OTR function in a manner similar to exogenous treatment. This may also explain, at least in part, long-term non-genomic effects of even single events, such as handling or maternal separation, which if they cause a change in endogenous levels of OT, stimulate a positive feedback on OT production.

The postnatal development of the oxytocinegric system may at least in part be due to the role of OT during pregnancy and labor in eutherian mammals. During pregnancy, circulating levels of OT are high and, depending upon the species, peak toward the end of pregnancy. At parturition there are additional pulses of OT, which facilitate uterine contractions. If OTR was present in the late-term fetus then circulating levels could have a significant impact on the organization of the fetal brain. Although limited in its scope, a comparative examination of ontogeny of the oxytocinergic system in the Brazilian opossum, a South American marsupial, supports this hypothesis. The ontogeny of the vasopressinergic and oxytocingeric systems are similar to eutherian mammals with AVP immunoreactivity occurring as early as E12 (Iqbal and Jacobson, 1995a), while OT-IR appears in the median eminence P1 with production occurring in the PVN and SON and posterior pituitary between P3 and P5 (Iqbal and Jacobson, 1995b). The development of the two systems in a primitive marsupial suggests that the developmental timing of the two systems is adaptive and may have evolved in the ancestral state. It also suggests birth is a critical aspect in the ontogeny of the oxytocinergic system. Compared to eutherian mammals birth in marsupials occurs at a significantly earlier stage of development. However, despite this chronological difference development of both systems occurs within a similar timeframe, suggesting that changes associated with parturition trigger development of the oxytocinergic system.

4.5.2 Vasopressinergic system

While Boer et al. (1994) speculated on an organizational effect of vasopressin there is little experimental evidence indicating pre- or postnatal organizational effects of AVP on the mechanisms regulating social behavior. At least two factors may have acted to limit study. First, as previously discussed, peripheral AVP plays a major role in water balance therefore manipulation of AVP can have significant impacts on physiological responses, which in turn may result in significant changes in behavior. Second, very early in the study of the role of AVP, in contrast to OT, it was discovered that not only are adults, but also the pre- and post-natal effects of AVP are testosterone dependent. Neonatal castration reduced the number of cells producing AVP in the brain and replacement restored it (DeVries et al., 1983). Testosterone has a direct effect on the expression of AVP mRNA in both the medial amygdala and the BST (Wang, 1994), two regions of the brain that are part of the social neural network and play a critical role in the expression of social behavior (Newman, 1999). Prenatal treatment with flutamide, an androgen receptor antagonist, eliminated the dependence of adult male rats on vasopressin for the formation of social memory and recognition (Axelson et al., 1999). Testosterone has the potential to organize the male vasopressinergic system from the onset as the vasopressinergic system begins embryonic development at the same time as testosterone increases in the fetal male. The steroidal regulation of AVP may also explain the sexually dimorphic role of AVP, with AVP playing a more significant role in male behavior (Cushing et al., 2001; Insel and Young, 2001). Combined, these studies suggest that many of the early developmental effects of AVP are under the regulation of testosterone, especially in males, and may have helped focus research on the role of testosterone on the vasopressinergic system rather than the organizational effects of AVP. Despite the lack of empirical evidence, given the close structural relationship of AVP to its sister nonapeptide OT, which has organizational effects, and the importance of AVP in regulating social behavior, it is probable that AVP does have organizational effects. The question is not only whether AVP has organizational effects, but also if they are independent of testosterone?

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The organizational effects of AVP are a potentially significant area of research, but only time, and possibly funding, will tell.

4.6 Implications in social deficit disorders

Given the critical role OT and AVP play in the regulation of social behavior, it is not surprising that there is intense interest in, and rapidly expanding research programs to understand, the role of OT and AVP not only as underlying mechanisms of, but also for possible treatments of social deficit disorders. The potential importance/role of neuropeptides in social deficit disorders is discussed in detail Part III – Human studies; and there are a number of comprehensive reviews that include information about neuropeptides and social deficit disorders (e.g., Heinrich et al., 2009; Ryckmans, 2010). Some of which review specific conditions, such as autism (Chapters 19 and 20), schizophrenia, depression, and ADHD (see Carter, 2007; Marazziti and Dell'Osso, 2008; Neuhaus et al., 2010). The goal of this section is to highlight specifically some of the findings as associated with the early onset of social deficit disorders and the role of OT and AVP.

Literature reviews of the neurobiology of social deficit disorders and empirical human studies indicate that the same factors, the organizational and epigenetic effects of OT and AVP, as discussed in this chapter, may play a significant role in the expression of social deficit disorders (Carter, 2007; Gregory et al., 2010; Insel, 2010). It has also been hypothesized that the sexually dimorphic expression of social deficit disorders may be explained, at least in part, by the differential effects of AVP and OT. For example, it has been argued that the sexually dimorphic actions of AVP may increase the probability that a male will develop autism and that OT may potentiate or buffer the effects of AVP (Carter, 2007). In fact, individuals with ASD have significantly higher levels of AVP (Boso et al., 2007), while autistic children have reduced levels of OT (Modahl et al., 1998; Green et al., 2001). Differential receptor expression may also play a role, as polymorphisms in the OTR gene have been correlated with autism (Jacob et al., 2007) and attention deficit and hyperactivity disorder (Park et al., 2010).

In adults, low circulating levels of OT have been associated with autism and other social deficit disorders and the use of OT has been shown to temporarily reduce the symptomatic expression of social deficits (Hollander et al., 2007). It is tempting to speculate that if early onset conditions, such as autism, are subject to organizational influences of neuropeptides then early intervention may prevent permanent, or at least limit long-term, changes within the CNS, as opposed to temporarily reducing symptoms. One case in point is Prader–Willi Syndrome, which is associated with low circulating levels of OT (Hoybye, 2004) and fewer OT neurons (Swaab et al., 1995). In a potential mouse model for Prader–Willi Syndrome, neonatal feeding deficits were eliminated with a single injection of OT at birth (Schaller et al., 2010). The findings from studies of social deficit disorders underscore and emphasize the take home message of this chapter is the significance of understanding the roles of critical periods for the organizational effects of OT and AVP.

REFERENCES

Altstein, M. and Gainer, H. (1988). Differential biosynthesis and posttranslational processing of vasopressin and oxytocin in rat-brain during embryonic and postnatal-development. *Journal of Neuroscience*, 8, 3967–3977.

Axelson, J. F., Smith, M., and Duarte, M. (1999). Prenatal flutamide treatment eliminates the adult male rat's dependency upon vasopressin when forming social–olfactory memories. *Hormones and Behavior*, 36, 109–113.

Bales, K. L., Abdelnabi, M., Cushing, B. S., Ottinger, and M. A., Carter, C. S. (2004b). Effects of neonatal oxytocin manipulations on male reproductive potential in prairie voles. *Physiology and Behavior*, 81, 519–526.

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*). *Hormones and Behavior*, 44, 178–184.

Bales, K. L. and Carter, C. S. (2003b). Developmental exposure to oxytocin facilitates partner preferences in male

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prairie voles (*Microtus ochrogaster*). *Behavioral Neuroscience*, 117, 854–859.

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

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

Bloch, B., Guitteny, A. F., Chouham, S., Mougin, C., Roget, A., and Teoule R. (1990). Topography and ontogeny of the neurons expressing vasopressin, oxytocin, and somatostatin genes in the rat-brain – an analysis using radioactive and biotinylated oligonucleotides. *Cellular and Molecular Neurobiology*, 10, 99–112.

Boer, G. J. (1985). Vasopressin and brain-development – studies using the Brattleboro rat. *Peptides*, 6, 49–62.

Boer, G. J. (1993). Chronic oxytocin treatment during late gestation and lactation impairs development of rat offspring. *Neurotoxiology and Teratology*, 15, 383–389.

Boer, G. J., Quak, J., DeVries, M. C., and Heinsbroek, R. P. W. (1994). Mild sustained effects of neonatal vasopressin and oxytocin treatment on brain growth and behavior of the rat. *Peptides*, 15, 229–236.

Boer, G. J., VanHeerikhuize, J., and Vanderwoude, T. P. (1988). Elevated serum oxytocin of the vasopressin-deficient brattleboro rat is present throughout life and is not sensitive to treatment with vasopressin. *ACTA Endocrinology*, 117, 442–450.

Boso, M., Emanuele, E., Politi, P., Pace A., Arra, M., di Nemi, S. U., and Barale, F. (2007). Reduced plasma apelin levels in patients with autistic spectrum disorder. *Archives of Medical Research*, 38, 70–74.

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

Carter, C. S., Boone, E. M., Pournajafi-Nazarloo, H., and Bales, K. L. (2009). Consequences of early experiences and exposure to oxytocin and vasopressin are sexually dimorphic. *Developmental Neuroscience*, 31, 332–341.

Carter, C. S., Witt, D. M., Schnieder, J., Harris, Z. L., and Volkening, D. (1987). Male stimuli are necessary for female sexual-behavior and uterine growth in prairie voles (*Microtus-ochrogaster*). *Hormones and Behavior*, 21, 74–82.

Cassoni, P., Catalano, M. G., Sapino, A., Marrocco, T., Fazzari, A., Bussolati, G., and Fortunati, N. (2002). Oxytocin modulates estrogen receptor alpha expression and function in MCF7 human breast cancer cells. *International Journal of Oncology*, 21, 375–378.

Cassoni, P., Sapino, A., Fortunati, N., Munaron, L., Chini, B., and Bussolati, G. (1997). Oxytocin inhibits the proliferation of MDA-MB231 human breast-cancer cells via cyclic adenosine monophosphate and protein kinase A. *International Journal of Cancer*, 72, 340–344.

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology*, 29, 386–397.

Champagne, F. A., Weaver, I. C. G., Diorio, J., Sharma, S., and Meaney, M. J. (2003). Natural variations in maternal care are associated with estrogen receptor a expression and estrogen sensitivity in the medial preoptic area. *Endocrinology*, 144, 4720–4724.

Chen, X. F., Chen, Z. F., Liu, R. Y., and Du, Y. C. (1988). Neonatal administration of a vasopressin analog (DDVAP) and hypertonic saline enhance learning-behavior in rats. *Peptides*, 4, 717–721.

Chen, Q., Schreiber, S. S., and Brinton, R. D. (2000). Vasopressin and oxytocin receptor mRNA expression during rat telencephalon development. *Neuropeptides*, 34, 173–180.

Choleris, E., Gustafsson, J. A., Korach, K. S., Muglia, L. J., Pfaff, D. W., and Ogawa, S. (2003). An estrogen-dependent four-gene micronet regulating social recognition: A study with oxytocin and estrogen receptor-alpha and -beta knockout mice. *Proceedings of the National Academy of Sciences USA*, 100, 6192–6197.

Choy, V. J. and Watkins, W. B. (1979). Maturation of the hypothalamo-neurohypophyseal system .1. localization of neurophysin, oxytocin and vasopressin in the hypothalamus and neural lobe of the developing rat-brain. *Cell and Tissue Research*, 197, 325–336.

Cushing, B. S. and Carter, C. S. (1999). Prior exposure to oxytocin mimics the effects of social contact and facilitates sexual behavior in females. *Journal of Neuroendocrinology*, 11, 765–769.

Cushing, B. S. and Kramer, K. M. (2005). Mechanisms underlying epigenetic effects of early social experience: the role of neuropeptides and steroids. *Neuroscience and Biobehavioral Reviews*, 29, 1089–1115.

Cushing, B. S., Levine, K., and Cushing, N. L. (2005). Neonatal manipulations of oxytocin affect reproductive behavior and reproductive success of adult female prairie voles (*Microtus ochrogaster*). *Hormones and Behavior*, 47, 22–28.

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Cushing, B. S., Martin, J. O., Young, L. J., and Carter, C. S. (2001). The effects of neuropeptides on partner preference formation are predicted by habitat. *Hormones and Behavior*, 39, 48–58.

Cushing, B. S., Razzoli, M., Murphy, A. Z., Epperson, P. D., and Hoffman, G. E. (2004). Intraspecific variation in estrogen receptor alpha and the expression of male behavior in two populations of prairie voles. *Brain Research*, 1016, 247–254.

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

DeVries, G. J., Best, W., and Sluiter, A. A. (1983). The influence of androgens on the development of a sex difference in the vasopressinergic innervation of the rat lateral septum. *Developmental Brain Research*, 8, 377–380.

Eaton, J. L., Roche, L., Nguyen, K. N., Troyer, E., Papademetriou, E., Cushing, B. S., and Raghanti, M. A. (2011). *Developmental Psychobiology*, online May 18.

Feldman, R., Gordon, I., and Zagoory-Sharon, O. (2010). The cross-generation transmission of oxytocin in humans. *Hormones and Behavior*, 58, 669–676.

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 Insel, T. R. (2002). Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: gender differences. *Journal of Neuroendocrinology*, 14, 349–353.

Green, L., Fein, D., Modahl, C., Feinstein, C., Waterhouse, L., and Morris, M. (2001). Oxytocin and autistic disorder: Alterations in peptide forms. *Biological Psychology*, 50, 609−613.

Gregory, S. G., Connelly, J. J., Towers, A. J., Johnson, J., Biscocho, D., Markunas, C. A., Lintas, C., Abramson, R. K., Wright, H. H., Ellis, P., Langford, C. F., Worley, G., Delong, G. R., Murphy, S. K., Cuccaro, M. L., Persico, A., and Pericak-Vance, M. A. (2009). Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. *BMC Medicine*, 7, 62.

Grippo, A. J., Cushing, B. S., and Carter, C. S. (2007). Depression-like behavior and stressor-induced neuroendocrine activation in female prairie voles exposed to chronic social isolation. *Psychological Medicine*, 69, 149–157.

Grippo, A. J., Trahanas, D. M., Zimmerman, R. R., Porges, S. W., and Carter, C. S. (2009). Oxytocin protects against negative behavioral and autonomic consequences of long-term social isolation. *Psychoneuroendocrinology*, 34, 1542–1553.

Heinrichs, M., von Dawans, B., and Domes, G. (2009). Oxytocin, vasopressin, and human social behavior. *Frontiers in Neuroendocrinology*, 30, 547–558.

Hnatczuk, O. C., Lisciotto, C. A., DonCarlos, L. L., Carter, C. S., and Morrell, J. I. (1994). Estrogen and progesterone receptor immunoreactivity (ER-IR and PR-IR) in specific brain areas of the prairie vole *(Microtus ochrogaster)* is altered by sexual receptivity and genetic sex. *Journal of Neuroendocrinology*, 6, 89–100.

Holst, S., Uvnas-Moberg, K., and Petersson, M. (2002). Postnatal oxytocin treatment and postnatal stroking of rats reduce blood pressure in adulthood. *Autonomic Neuroscience: Basic and Clinical*, 99, 85–90.

Hollander, E., Bartz, J., Chaplin, W., Phillips, A., Sumner, J., Soorya, L., Anagnostou, E., and Wasserman, S. (2007). Oxytocin increases retention of social cognition in autism. *Biological Psychology*, 61, 498–503.

Hoybye, C. (2004). Endocrine and metabolic aspects of adult Prader–Willi syndrome with special emphasis on the effect of growth hormone treatment. *Growth Hormone & IGF Research*, 14, 1–15.

Insel, T. R. (2010). The challenge of translation in social neuroscience: a review of oxytocin, vasopressin, and affiliative behavior. *Neuron*, 65, 768–779.

Insel, T. R. and Winslow, J. T. (1991). Central administration of oxytocin modulates the infant rats response to social-isolation. *European Journal of Pharmacology*, 203, 149–152.

Insel, T. R. and Young, L. J. (2001). The neurobiology of attachment. *Nature Review Neuroscience*, 2, 129–136.

Iqbal, J. and Jacobson, C. D. (1995a). Ontogeny of arginine vasopressin-like immunoreactivity in the Brazilian opossum brain. *Developmental Brain Research*, 89, 11–32.

Iqbal, J. and Jacobson, C. D. (1995b). Ontogeny of oxytocin-like immunoreactivity in the Brazilian opossum brain. *Developmental Brain Research*, 90, 1–16.

Ivell, R. and Walther, N. (2002). The role of sex steroids in the oxytocin hormone system. *Molecular and Cellular Endocrinology*, 151, 95–101.

Jacob, S., Brune, C. W., Carter, C. S., Leventhal, B. L., Lord, C., and Cook, E. H. (2007). Association of the oxytocin receptor gene (OXTR) in Caucasian children and adolescents with autism. *Neuroscience Letters*, 417, 6–9.

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Jia, R., Tai, F., An, S., Broders, H., and Sun, S. (2008). Neonatal manipulation of oxytocin influences the partner preference in Mandarin voles (*Microtus mandarinus*). *Neuropeptides*, 42, 525–533.

Jing, X., Ratty, A. K., and Murphy, D. (1998). Ontogeny of the vasopressin and oxytocin RNAs in the mouse hypothalamus. *Neuroscience Research*, 30, 343–349.

Kleiman, D. G. (1977). Monogamy in mammals. *Quarterly Review of Biology*, 52, 39–69.

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

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 Neuroscience*, 8, 71.

Lukas, M., Bredewold, R., Neumann, I. D., and Veenema, A. H. (2010). Maternal separation interferes with developmental changes in brain vasopressin and oxytocin receptor binding in male rats. *Neuropharmacology*, 58, 78–87.

Marzona, L., Arletti, R., Benelli, A., Sena, P., and DePol, A. (2003). Effects of estrogens and oxytocin on the development of neonatal mammalian ovary. *In vivo*, 15, 271–279.

Marazziti, D. and Dell'Osso, M. C. (2008). The role of oxytocin in neuropsychiatric disorders. *Current Medicinal Chemistry*, 7, 698–704.

McCarthy, M. M., McDonald, C. H., Brooks, P. J., and Goldman, D. (1997). An anxiolytic action of oxytocin is enhanced by estrogen in the mouse. *Physiology and Behavior*, 60, 1209–1215.

Meyerson, B. J., Hoglund, U., Johansson, C., Blomqvist, A., and Ericson, H. (1988). Neonatal vasopressin antagonist treatment facilitates adult copulatory-behavior in female rats and increases hypothalamic vasopressin content. *Brain Research*, 437, 344–351.

Modahl, C., Green, L., Fein, D., Morris, M., Waterhouse, L., Feinstein, C., and Levin, H. (1998). Plasma oxytocin levels in autistic children. *Biological Psychology*, 43, 270−277.

Neuhaus, E., Beauchaine, T. P., and Bernier, R. (2010). Neurobiological correlates of social functioning in autism. *Clinical Psychology Review*, 30, 733–748.

Newman, S. W. (1999). The medial extended amygdala in male reproductive behavior – A node in the mammalian social behavior network. *Annals of the New York Academy of Science*, 877, 242–257.

Noonan, L. R., Continella, G., and Pedersen, C. A. (1989). Neonatal administration of oxytocin increases novelty-induced grooming in the adult-rat. *Pharmacology Biochemistry and Behavior*, 33, 555–558.

Oreland, S., Gustafsson-Ericson, L., and Nylander, I. (2010). Short- and long-term consequences of different early environmental conditions on central immunoreactive oxytocin and arginine vasopressin levels in male rats. *Neuropeptides*, 44, 391–398.

Parent, A. S., Rasier, G., Matagne, V., Lomniczi, A., Lebrethon, M. C., Gerard. A., Ojeda, S. R., and Bourguignon, J. P. (2008). Oxytocin facilitates female sexual maturation through a glia-to-neuron signaling pathway. *Endocrinology* 149, 1358–1365.

Park, J., Willmott, M., Vetuz, G., Toye, C., Kirley, A., Hawi, Z., Brookes, K. J., Gill, M., and Kent, L. (2010). Evidence that genetic variation in the oxytocin receptor (OXTR) gene influences social cognition in ADHD. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 34, 697–702.

Perry, A. N., Paramadilok, A., and Cushing, B. S. (2009). Neonatal oxytocin alters subsequent estrogen receptor alpha protein expression and estrogen sensitivity in the female rat. *Behavioral Brain Research*, 205, 154–161.

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

Pournajafi-Nazarloo, H., Papademeteriou, E., Saadat, H., Partoo, L., and Cushing, B. S. (2007b). Modulation of cardiac oxytocin receptor and estrogen receptor alpha mRNAs expression following neonatal oxytocin treatment. *Endocrine*, 31, 154–160.

Pournajafi-Nazarloo, H., Perry, A., Partoo, L., Papademeteriou, E., Azizi, F., Carter, C. S., and Cushing, B. S. (2007c). Neonatal oxytocin treatment modulates oxytocin receptor, atrial natriuretic peptide, nitric oxide synthase and estrogen receptor mRNAs expression in rat heart. *Peptide*, 28, 1170–1177.

Roberts, R. L., Williams, J. R., Wang, A. K., and Carter, C. S. (1998). Cooperative breeding and monogamy in prairie voles: Influence of the sire and geographical variation. *Animal Behaviour*, 55, 1131–1140.

Ryckmans, T. (2010). Modulation of the vasopressin system for the treatment of CNS diseases. *Current Opinions in Drug Discovery and Development*, 13, 538–547.

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Schaller, F., Watrin, F., Sturny, R., Massacrier, A., Szepetowski, P., and Muscatelli, F. (2010). A single postnatal injection of oxytocin rescues the lethal feeding behaviour in mouse newborns deficient for the imprinted Magel2 gene. *Human Molecular Genetics*, 19, 4895–4905.

Schank, J. C. (2009). Early locomotor and social effects in vasopressin deficient neonatal rats. *Behavioral Brain Research*, 197, 166–177.

Shapiro, L. E. and Insel, T. R. (1989). Ontogeny of oxytocin receptors in rat forebrain – A quantitative study. *Synapse*, 3, 259–266.

Shepard, K. N., Vasiliki Michopoulos, V., Toufexis, D. J., and Wilson, M. E. (2009). Genetic, epigenetic and environmental impact on sex differences in social behavior. *Physiology and Behavior*, 97, 157–170.

Snijdewint, F. G. M. and Boer G. J. (1988). Neonatal treatment with vasopressin antagonist dp[tyr(me)2]avp, but not with vasopressin antagonist d(ch2)5[tyr(me)2]avp, inhibits body and brain-development and induces polyuria in the rat. *Neurotoxicological Teratology*, 10, 321–325.

Sohlström, A., Olausson, H., Brismar, K., and Uvnäs-Moberg, K. (2002). Oxytocin treatment during early life influences reproductive performance in *ad libitum* fed and food-restricted female rats. *Biology of the Neonate*, 81, 132–138.

Stone, A. L. and Bales, K. L. (2010). Intergenerational transmission of the behavioral consequences of early experience in prairie voles. *Behavioral Process*, 84, 732–738.

Stribley, J. M. and Carter, C. S. (1999). Developmental exposure to vasopressin increases aggression in adult prairie voles. *Proceedings of the National Academy of Sciences USA*, 96, 12601–12604.

Swaab, D. F., Purba, J. S., and Hofman, M. A. (1995). Alterations in the hypothalamic paraventricular nucleus and its oxytocin neurons (putative satiety cells) in Prader–Willi syndrome: a study of five cases. *Journal of Clinical Endocrinology and Metabolism*, 80, 573–579.

Tribollet, E., Goumaz, M., Raggenbass, M., Dubois-Dauphin, M., and Dreifuss, J. J. (1991). Early appearance and transient expression of vasopressin receptors in the brain of rat fetus and infant. An autoradiographical and electrophysiological study. *Developmental Brain Research*, 58, 13–24.

Todeschin, A. S., Winkelmann-Duarte, E. C., Jacob, M. H. V., Aranda, B. C. C., Jacobs, S., Fernandes, M. C., Ribeiro, M. F. M., Sanvitto, G. L., and Lucion, A. B. (2009). Effects of neonatal handling on social memory, social interaction, and number of oxytocin and vasopressin neurons in rats. *Hormones and Behavior*, 56, 93–100.

Uvnäs-Moberg, K., Alster, P., Petersson, M., Sohlström, A., and Björkstrand, E. (1998). Postnatal oxytocin injections cause sustained weight gain and increased nociceptive thresholds in male and female rats. *Pediatric Research*, 43, 1–5.

Varlinskaya, E. I., Petrov, E. S. Robinson, S. R., and Smotherman, W. P. (1994). Behavioral-effects of centrally administered arginine-vasopressin in the rat fetus. *Behavioral Neuroscience*, 108, 395–409.

Vorbrodt, A. W. (1993). Morphological evidence of the functional polarization of brain microvascular epithelium. In *The Blood–Brain Barrier*, Raven Press, Ltd., New York, NY: W.M. Pardridge, pp. 137–164.

Wang, Z. X. (1994). Testosterone effects on development of vasopressin messenger-RNA expression in the bed nucleus of the stria terminalis and medial amygdaloid nucleus in male-rats. *Developmental Brain Research*, 79, 147–150.

Winslow, J. T. and Insel, T. R. (1993). Effects of central vasopressin administration to infant rats. *European Journal of Pharmacology*, 233, 101–107.

Withuhn, T., Kramer, K. M., and Cushing, B. S. (2003). Early exposure to oxytocin affects the age of vaginal opening and first estrus in female rats. *Physiology and Behavior*, 8, 135–138.

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

Yamamoto, Y., Cushing, B. S., Kramer, K. M., Epperson, P., 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.

Yoshimura, R., Kimura, T., Watanabe, D., and Kiyama, H. (1996). Differential expression of oxytocin receptor mRNA in the developing rat brain. *Neuroscience Research*, 24, 291–304.

Young, E., Carter, C. S., Cushing, B. S., and Caldwell, J. D. (2005). Neonatal manipulation of oxytocin alters oxytocin levels in the pituitary of adult rats. *Hormone and Metabolic Research*, 37, 397–401.