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Developmental effects of oxytocin on neural activation and neuropeptide release in response to social stimuli

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Abstract

Previous studies have revealed that the neuropeptide hormone oxytocin (OT) has developmental effects on subsequent social behavior and on mechanisms underlying social behavior such as OT neurons and estrogen receptor α. This suggests that OT might also have developmental effects on neural responses to social stimuli. This was tested in socially monogamous prairie voles (*Microtus ochrogaster*) by manipulating OT on the first day of life and then assessing the response to a heterosexual pairing in adulthood. The response to cohabitation was assessed by quantifying neural activation in regions of the brain associated with sociosexual behavior and anxiety using c-Fos immunoreactivity. Additionally, immunocytochemistry was used to label OT and vasopressin neurons and plasma was assayed for both neuropeptides. Treatment effects were evident in females, but not in males. Blockade of OT receptors with an OT antagonist on the first day of life resulted in neural activation of the central amygdala in response to a pairing with a novel male in adulthood. The central amygdala does not normally express c-Fos after a heterosexual pairing in reproductively naïve prairie voles. Treatment effects also were observed in vasopressin immunoreactivity in the SON with OT-treated females showing a decrease.

Keywords: c-Fos; Central amygdala; Development; *Microtus*; Monogamy; Neural activation; Oxytocin; Social bonds; Vasopressin; Vole

Introduction

A number of recent studies have demonstrated that manipulation of the neuropeptide oxytocin (OT) during the early postnatal period can have long-lasting effects on the expression of social and sociosexual behavior (Bales et al., 2004; Cushing et al., 2005; Kramer et al., 2003; reviewed in Carter, 2003). Neonatal manipulation of OT also affects the mechanisms that regulate these behaviors. In prairie voles (*Microtus ochrogaster*), neonatal manipulation of OT increased the number of OT neurons in the paraventricular nucleus of the hypothalamus (PVN), a region that produces most of the centrally released OT (Yamamoto et al., 2004). Neonatal manipulation of OT also altered the expression of estrogen receptor α in two regions of the brain that regulate sociosexual behavior, the medial preoptic area (MPOA) and the ventromedial nucleus of the hypothalamus (VMN) (Yamamoto et al., in press). These results suggest that manipulation of OT neonatally not only affects the organization of the brain, but that it also may affect the neural response to social stimuli. Therefore, the goal of this study was to test the hypothesis that neonatal manipulation of OT alters neural activation in response to social stimuli.

This hypothesis was tested by examining neuronal activation in prairie voles in response to cohabitation with a member of the opposite sex. Neural activation was assessed by quantifying the expression of the immediate-early gene c-*fos*. Prairie voles are a good model to study the effects of OT on response to social interactions for several reasons. Prairie voles are socially monogamous and form long-term pair bonds (Getz et al., 1981; Thomas and Birney, 1979). Females do not undergo a spontaneous estrous cycle; prolonged contact (24 h or more) with a novel male is necessary to induce behavioral and physiological estrus

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(Carter et al., 1987; Dluzen et al., 1981). Thus, results can be interpreted as a response to social stimuli without being confounded by neural and physiological responses to hormonal estrus and sexual receptivity. Studies using the expression of c-Fos as a marker of neuronal activation have shown that changes in neuronal activity occur shortly after pairing with a novel individual (Cushing et al., 2003a; Lim and Young, 2004, Schwab et al., 2004). The response to cohabitation is sexually dimorphic and varies depending upon whether an individual is paired with a member of the opposite sex or the same sex (Cushing et al., 2003a). Finally, because the regions of the brain that are activated in response to initial social contact with a member of the opposite sex have been described (Cushing et al., 2003a; Lim and Young, 2004; Schwab et al., 2004), specific predictions can be made and results can be compared against previous findings.

In rodents, the first phase of contact with a novel conspecific typically involves anogenital sniffing and subsequent processing by the vomeronasal organ and the accessory olfactory bulb (Barber and Raisman, 1974; Dudley et al., 1992; Fiber et al., 1993). The accessory olfactory bulb has connections to the medial amygdala (MeA) and the bed nucleus of the stria terminalis (BNST), both of which have projections to the MPOA and the VMN (Kevetter and Winans, 1981). The MeA, BNST, MPOA, and VMN are considered a part of an extended network that mediates social and sexual behavior (Newman, 1999). Additionally, studies using c-Fos expression as a marker of neural activation have shown that these regions are activated in prairie voles after varying periods of cohabitation (Curtis and Wang, 2003; Cushing et al., 2003a; Lim and Young, 2004). However, additional regions also express c-Fos depending upon the time since cohabitation and the sex of the individual (Hazelton et al., 2003). For example, a 2-h cohabitation resulted changes in c-Fos immunoreactivity (IR) in the ventral pallidum and nucleus accumbens (Lim and Young, 2004), but not after 1 h (Cushing et al., 2003a) or after 6 h of cohabitation (Curtis and Wang, 2003). Additionally, males paired with an unrelated male displayed an increase in c-Fos in the central amygdala (CeA) after 1 h (Cushing et al., 2003a). The CeA is associated with fear response and anxiety-related behaviors (Gray and Bingaman, 1996), suggesting that cohabitation with a novel individual of the same sex may be more stressful for males. This is interesting in light of the current study as OT is anxiolytic (Amico et al., 2004; Bale et al., 2001; McCarthy et al., 1996; Windle et al., 1997). Not only does the CeA have OT receptors (OTR) (Gimpl and Fahrenholz, 2001), a recent study suggests that the anxiolytic effects of OT may be mediated by inhibition of specific neuronal populations in the CeA (Huber et al., 2005). Individual and sex differences in the CeA may help to explain differential responses to novel stimuli.

Several predictions can be made about the effects of neonatal manipulation of OT on c-Fos expression. (1) Because neonatal blockade of OTR generally results in a reduction of social behavior (Bales et al., 2004; Kramer et al., 2003), treatment with OTA early in life was expected to result in reduced c-Fos expression in brain regions associated with pair bonding. OTA is not expected to prevent a pair bond from forming but may alter how a pair bond is formed, possibly through effects on neural processing or changes in brain regions that are activated during initial encounters. (2) OT treatment may enhance the response to a member of the opposite sex. If this were occurring, we might predict that areas, such as the nucleus accumbens, which do not show increased c-Fos until after 2 h of mating (Lim and Young, 2004) would respond during the first hour. (3) Neonatal manipulation of OT could stimulate neuronal activation in other regions of the brain. Specifically, OT is anxiolytic (Mantella et al., 2003; Neumann et al., 2000; Windle et al., 1997); therefore, inhibition of the effects of OT could increase the stress response to a novel conspecific by affecting areas such as the CeA. (4) Responses are predicted to be sexually dimorphic. Neonatal manipulations of OT have produced a sexually dimorphic response in other studies examining a wide variety of dependent variables (Bales and Carter, 2003; Kramer et al., 2003; Yamamoto et al., 2004).

In addition to quantifying c-Fos, we also assessed the effects of neonatal treatment on central levels of OT and a related neuropeptide hormone also involved in social behavior, arginine vasopressin (AVP), using immunocytochemistry. Plasma OT and AVP were measured after the cohabitation period to provide context for any treatment effects on immunoreactivity for either neuropeptide in the supraoptic nucleus of the hypothalamus (SON) or in the PVN.

Materials and methods

Husbandry

Subjects were prairie voles originating from a population trapped near Urbana, IL. All animals were maintained on a 14:10 light:dark cycle and provided with Purina high-fiber rabbit chow and water ad libitum. Breeding pairs were checked daily for litters. On the day of birth (D1), pups were sexed, treated (see below), and marked for identification. Because prairie voles have only 6 nipples and feeding competition could introduce a confounding source of error, litters in excess of 6 pups were culled to 6. At 21 days of age, pups were weaned and housed in same-sex sibling pairs until testing between 60 and 90 days of age. All subjects were sexually naïve when tested. All husbandry and experimental procedures were approved by the ACUC at the University of Illinois at Chicago and comply with the guidelines in the National Institutes of Health *Guidelines for the Care and Use of Animals*.