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OXYTOCIN AND VASOPRESSIN IMMUNOREACTIVE STAINING IN THE BRAINS OF BRANDT'S VOLES (*LASIOPodomys BRANDTII*) AND GREATER LONG-TAILED HAMSTERS (*TSCHERSKIA TRITON*)

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Abstract

Immunoreactive (ir) staining of the neuropeptides oxytocin (OT) and vasopressin (AVP) was performed in the brains of Brandt's voles (*Lasiopodomys brandtii*) and greater long-tailed hamsters (*Tscherskia triton*)—two species that differ remarkably in social behaviors. Social Brandt's voles had higher densities of OT-ir cells in the medial preoptic area (MPOA) and medial amygdala (MeA) as well as higher densities of AVP-ir cells in the lateral hypothalamus (LH) compared to solitary greater long-tailed hamsters. In contrast, the hamsters had higher densities of OT-ir cells in the anterior hypothalamus (AH) and LH and higher densities of AVP-ir cells in the MPOA than the voles. OT-ir and AVP-ir fibers were also found in many forebrain areas with subtle species differences. Given the roles of OT and AVP in the regulation of social behaviors in other rodent species, our data support the hypothesis that species-specific patterns of central OT and AVP pathways may underlie species differences in social behaviors. However, despite a higher density of OT-ir cells in the paraventricular nucleus of the hypothalamus (PVN) in females than in males in both species, no other sex differences were found in OT-ir or AVP-ir staining. These data failed to support our prediction that a sexually dimorphic pattern of neuropeptide staining in the brain is more apparent in Brandt's voles than in greater long-tailed hamsters.

Keywords

medial preoptic area; anterior hypothalamus; medial amygdala; solitary; social behavior

Species comparisons are a powerful tool used in experimental biology to study animal behavior and its underlying neural mechanisms. Using a comparative approach, remarkable differences have been found in several types of social behaviors, and these differences, when grouped together, constitute general life strategies. Species that follow a monogamous life strategy, for example, usually exhibit high levels of social affiliation among individuals, biparental care for their offspring, and selective aggression towards unfamiliar conspecifics (Kleiman, 1977; Dewsbury, 1987). In contrast, non-monogamous species tend to be solitary, less affiliative, and more aggressive. Such species differences in behavior may reflect

evolutionary pressure and/or a specific adaptation to the environment. In addition, such differences may indicate potential underlying species differences in the central nervous system.

Several elegant rodent models have been developed to examine species-specific behaviors and underlying neural patterns. By comparing monogamous prairie (*Microtus ochrogaster*) and pine (*M. pinetorum*) voles with promiscuous montane (*M. montanus*) and meadow (*M. pennsylvanicus*) voles, differences have been found in several patterns of social behaviors, including pair bonding, parental care, and selective aggression (Getz et al., 1981; McGuire and Novak, 1984, 1986; Oliveras and Novak, 1986; Shapiro and Dewsbury, 1990), and such differences in behaviors have been correlated with species differences in central neurotransmitter systems including oxytocin (OT), vasopressin (AVP), and dopamine (DA) (Insel and Shapiro, 1992; Insel et al., 1994; Wang et al., 1997; Aragona et al., 2006; Young et al., 2008). Further, pharmacological and molecular manipulations in monogamous and promiscuous vole species have shown that OT, AVP, and DA play important roles in regulating social behaviors associated with a monogamous life strategy (Winslow et al., 1993; Williams et al., 1994; Lim et al., 2004; Aragona et al., 2006). A similar comparative approach has also been applied to the study of social behavior and its underlying neural mechanisms in mice. Monogamous California mice (*Peromyscus californicus*) are highly social and display biparental behavior whereas promiscuous male white-footed mice (*P. leucopus*) are primarily solitary during the breeding season and exhibit less paternal behavior (Madison et al., 1984; Bester-Meredith et al., 1999). It has been shown that these species differences in social behaviors are correlated with differences in the extra-hypothalamic AVP pathway, particularly the AVP pathway in the bed nucleus of the stria terminalis (BST) and the medial nucleus of the amygdala (MeA) (Bester-Meredith et al., 1999; Bester-Meredith and Marler, 2001, 2003). Furthermore, in a recent study in two related South American rodent species, the social colonial tuco-tuco (*Ctenomys sociabilis*) and solitary Patagonian tuco-tuco (*Ctenomys haigi*), species differences were found in OT and AVP receptor distributions in the brain, implicating OT and AVP in the behavioral differences noted between these species (Beery et al., 2008). Interestingly, in a study comparing several monogamous avian species (family *Estrildidae*), the receptor densities for vasotocin (an OT/AVP analog found in non-mammalian vertebrates) in the brain were related positively to sociality (Goodson et al., 2006).

OT and AVP are both nine amino-acid neuropeptides that are primarily synthesized in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus, transported into the posterior pituitary gland, and then released into the blood stream to mediate physiological functions in a variety of species (Brownstein et al., 1980). OT has been shown to play an important role in uterine contraction and milk letdown (Caldeyrobarcia and Poseiro, 1960; Young et al., 1996), whereas AVP regulates water re-absorption and blood pressure (Edmunds and West, 1962; Nielsen et al., 1995). However, OT or AVP synthesizing cells are also found in other brain areas, such as the anterior hypothalamus (AH), medial preoptic area (MPOA), BST and MeA (DeVries et al., 1985; Young and Gainer, 2003; Rosen et al., 2008). These cells project centrally into forebrain areas in which OT and AVP are released to regulate cognitive and behavioral functions, including parental behavior, pair bonding, social recognition, learning and memory (Dantzer et al., 1988; Ferguson et al., 2002; Caldwell et al., 2008). In addition, central AVP has been implicated in flank marking (a stereotypical scent marking behavior commonly displayed by hamsters) and aggression in males (Ferris et al., 1984, 1988; Everts et al., 1997). Interestingly, comparative studies have illustrated species differences in central OT and AVP pathways and their receptors and these differences have been implicated in species-specific life strategies and social behaviors (Insel and Shapiro, 1992; Insel et al., 1994; Bester-Meredith et al., 1999). Furthermore, sex differences have also been found in central OT and AVP

systems, implicating the role of those systems in regulating sex-specific cognitive and behavioral functions (De Vries and Panzica, 2006; Carter, 2007).

In the present study, we compared two local rodent species that belong to the *Cricetidae* family. Brandt's voles (*Lasiopodomys brandtii*) inhabit typical steppes in Inner Mongolia of China, Mongolia, and the region of Beigaer in Russia (Shi, 1988; Liu et al., 1994). They usually become sexually mature around 1.5 months of age, and their life span is about 1 year in the wild and 2.5 years in the laboratory (Zhang et al., 1998a). Brandt's voles are social animals that live in large family groups and display extensive social interactions among individuals (Yin and Fang, 1998; Wan et al., 1999; Chen and Shi, 2003). Sex differences in this species are found in both morphology and behavior, with males being larger and displaying higher levels of territorial defense than females (Hou and Yin, 1996; Yin and Fang, 1998; Chen and Shi, 2003). In contrast, greater long-tailed hamsters (*Tscherskia triton*) are distributed primarily in the farmland and wilderness of northern China, the region of Siberia in Russia, and Korea (Yang et al., 1996; Zhang et al., 1998b). They reach sexual maturity around 1.5 months of age, and their life span is about 1 year in the wild and 3–4 years in laboratory conditions (Zhang et al., 1998b). These hamsters live in solitude throughout the year and participate in limited social affiliation with conspecifics, except during breeding seasons (Zhang et al., 1999, 2001b). Males and females do not differ in their body sizes and both display similar levels of flank marking behavior and aggression (Zhang et al., 2001a; Wang et al., 2006). Although differences in social behaviors have been shown between the two species (Yin and Fang, 1998; Zhang et al., 1999, 2001b; Chen and Shi, 2003), we know little about the underlying neurochemical systems. As central OT and AVP systems have been implicated in species-specific patterns of social behaviors in other rodent species, we hypothesized that Brandt's voles and greater long-tailed hamsters differ in their OT and AVP systems which, in turn, may regulate their differences in social behaviors. In the current study, as the first step to test this hypothesis, we compared OT and AVP immunoreactive (ir) staining in the brains of these two species. We predicted that the two species may differ in the distribution pattern and regional quantity of OT and/or AVP staining, correlating with their differences in social behaviors. In addition, we hypothesized that sexually dimorphic patterns in OT and/or AVP staining would be more apparent in the Brandt's voles than in the greater long-tailed hamsters, as the former species demonstrates more sexually dimorphic behavioral patterns than the latter (Yin and Fang, 1998; Zhang et al., 2001a; Chen and Shi, 2003).

EXPERIMENTAL PROCEDURES

Subjects

Subjects were adult (3 months of age) male and female Brandt's voles (*Lasiopodomys brandtii*) and greater long-tailed hamsters (*Tscherskia triton*). Subjects were offspring of laboratory breeding colonies started with field captured animals and maintained in the Institute of Zoology at the Chinese Academy of Sciences in Beijing, China. All subjects were maintained in plastic cages (25×14×14 cm³ for Brandt's vole; 27×16×13 cm³ for greater long-tailed hamster) that contained wood shavings. Food and water were provided *ad libitum*. Brandt's voles were housed in same-sex groups, consisting of two to three individuals each, under a 16L:8D photoperiod (lights on 0500) while greater long-tailed hamsters were housed singly under a reversed 16L:8D photoperiod (lights on 1700). These housing conditions were chosen based upon the natural behaviors displayed by each species. Room temperature was maintained at 20±2 °C. All experimental procedures complied with the guidelines for animal use and care as stipulated by the Institute of Zoology at the Chinese Academy of Sciences.

Tissue preparation

Eight males and eight females of each species were deeply anesthetized with sodium pentobarbital (3 mg/100 g body weight, Sigma-Aldrich, St. Louis, MO, USA) and perfused through the ascending aorta with 0.1 M phosphate buffered solution (PBS, pH=7.2) followed by 4% paraformaldehyde in PBS. Brains were quickly removed, post-fixed in 4% paraformaldehyde for 12 h, and then stored in 30% sucrose in PBS. Coronal brain sections of 40- μ m thickness were cut on a cryostat. Two alternate sets of brain sections at 240- μ m intervals were processed for OT and AVP immunocytochemistry, respectively. An additional set of brain sections was processed for nissl staining.

OT and AVP immunocytochemistry

Floating brain sections were processed for OT or AVP immunocytochemistry using an established method (Wang et al., 1996). Briefly, brain sections were pre-treated with 0.5% NaBH₄, followed by 0.05% H₂O₂ in 0.05 M Tris–NaCl (pH=7.6), and then blocked in 10% normal goat serum (NGS) in Tris–NaCl with 0.5% Triton X-100 (Tris–Triton). Thereafter, sections were incubated in either anti-OT serum (1:20,000) or anti-AVP serum (1:40,000) (both from Bachem California, Inc., Torrance, CA, USA) in Tris–Triton with 2% NGS for 36 h at 4 °C and an additional 2 h at room temperature. Sections were then incubated with biotinylated goat-anti-rabbit (for OT, Vector Laboratories, Burlingame, CA, USA) or goat-anti-guinea pig (for AVP, Vector Laboratories) secondary antibody (1:300) in Tris–Triton for 2 h; ABC complex (Vector Laboratories) in Tris–NaCl for 90 min; and stained by 0.05% 3–3'-diaminobenzidine (Sigma-Aldrich) in Tris–NaCl with 0.009% H₂O₂. Sections were then mounted, air-dried and coverslipped. To reduce variability in the staining, brain sections from all subjects were processed concurrently. To control for antibody specificity, additional brain sections were incubated either in the absence of the primary antibody or with the primary antibody that was pre-treated with 50 μ M OT or AVP (both from Calbiochem, San Diego, CA, USA), respectively. In both situations, specific staining was eliminated or substantially reduced. Finally, one set of brain sections from each species was stained in 1% Cresyl Violet (Sigma-Aldrich) solution for 30 min, dehydrated, and coverslipped.

Data quantification and analysis

All slides were coded to conceal group identity. Slides from both species were inspected under a Nikon microscope to identify fore-brain regions with OT and/or AVP-ir staining. OT-ir cells were counted in the MPOA, AH (anterior part), posterior part of the AH (AHP), reticular thalamic nucleus (Rt), PVN, lateral hypothalamic area (LH), and MeA. For most brain regions, cell counts were obtained bilaterally from two anatomically matched, consecutive sections. However, for the intermediate part of the MPOA (MPOAi) in Brandt's voles and the Rt in both species, only one section was counted. All OT-ir cells were counted in each of the brain areas except for the AHP in which sampling grids were used. Since no differences were found in the numbers of OT-ir cells between the two hemispheres in each brain area examined, the cell counts were combined as a total number. A set of nissl stained sections from each species was used to measure the brain areas, which were then used to divide the number of OT-ir cells to get cell density per area. The density of OT-ir fibers was categorized using a semi-quantitative system distinguishing three categories: +, very few fibers; ++, a moderate fiber density; +++, a high fiber density, according to De Vries (De Vries et al., 1983). The density of OT-ir fibers was estimated in the MPOA, pariventricular hypothalamic nucleus (Pe), Rt, substantial innominata (SI), AH, BST, PVN, LH, SON and supraoptic decussation (SOX). Finally, quantification for AVP-ir cells and fiber densities were conducted using a similar method to the one described above for OT-ir.

It is important to note that the cell density, instead of cell number, in each brain area was used for data analysis because of the species differences in body mass. Data were analyzed by a two-way analysis of variance (ANOVA) with species and sex as between-subject variables. The criterion for significance was set at $P < 0.05$.

RESULTS

OT-ir staining

OT-ir stained cells were either present in dense clusters or scattered throughout many forebrain areas in both species. The morphology and overall staining pattern of OT-ir cells were quite similar to that reported in other rodent species (Sofroniew et al., 1979; Wang et al., 1996; Rosen et al., 2008). Very intense staining of OT-ir cells was found in the PVN (Fig. 1A, B) and SON while moderate clusters of OT-ir cells were found throughout the rostral–caudal extent of the MPOA, particularly in the MPOAa and MPOAi (Fig. 2). Furthermore, scattered OT-ir cells were found in many brain regions including the AH (Fig. 1C, D), LH (Fig. 1E, F), MeA (Fig. 1G, H), BST, median preoptic nucleus (MnPO), Pe, SI, nucleus of stria medullaris (SM), and ventromedial hypothalamus (VMH).

Species differences were found in the density of OT-ir cells in some brain areas. In the MPOA, Brandt's voles had a higher density of OT-ir cells than greater long-tailed hamsters ($F_{(1,28)} = 26.8$, $P < 0.01$; Fig. 3A). Interestingly, this species difference was seen in the MPOAa ($F_{(1,28)} = 48.9$, $P < 0.01$; Fig. 2A, B) and MPOAi ($F_{(1,28)} = 17.2$, $P < 0.01$; Fig. 2C, D and 3C), but the opposite pattern was found in the posterior part of the MPOA (MPOAp; $F_{(1,28)} = 32.5$, $P < 0.01$; Fig. 2E, F and 3C). Brandt's voles also had a higher density of OT-ir cells in the MeA ($F_{(1,28)} = 7.6$, $P < 0.01$) than greater long-tailed hamsters, whereas the latter had a higher density of OT-ir cells in the AH ($F_{(1,28)} = 4.6$, $P < 0.05$), LH ($F_{(1,28)} = 5.2$, $P < 0.05$) and PVN ($F_{(1,28)} = 66.2$, $P < 0.01$) than the former (Fig. 3A, B). OT-ir cells in the BST also showed different distribution patterns between species. Brandt's voles had cells in the anterior–medial (BSTMA), lateral (BSTL), ventral (BSTV) and postero-lateral–medial (BSTMPL) parts of the BST while greater long-tailed hamsters only had OT-ir cells in the BSTMPL. An overall sex difference was found in the PVN ($F_{(1,28)} = 7.0$, $P < 0.05$), in which females had a higher density of OT-ir cells than males (Table 1). No species or sex differences were found in the density of OT-ir cells in any other brain areas, and no species–sex interaction was found in any brain areas examined (Table 1).

OT-ir fibers were found throughout forebrain areas in both species. Some lightly-stained fibers were found in the LS. In the BST, scattered OT-ir fibers were found in the BSTMA but denser fibers were found in the BSTMPL (Fig. 4A, B). Less intense OT-ir fibers were found in the Pe, MPOA (Fig. 2), LH (Fig. 4E, F), Rt, and SI (Fig. 4I, J). High densities of OT-ir fibers emerged from the PVN and SON (Fig. 4M, N), and most of these fibers could be followed via the hypothalamo–neurohypophyseal tract into the median eminence, however, some traveled dorsolaterally toward the stria terminalis. The median eminence and SOX (Fig. 4Q, R) had the highest density of OT-ir fibers. Using the semi-quantitative measurement described above, Brandt's voles seemed to have a higher density of OT-ir fibers in the MPOA and BSTMPL than greater long-tailed hamsters (Table 2).

AVP-ir staining

The distribution patterns and morphology of AVP-ir cells were quite similar between the two species and compared to other species of rodents (Buijs, 1978; Sofroniew and Weindl, 1981; Wang, 1995). Dense clusters of AVP-ir cells were found in the suprachiasmatic nucleus (SCN), PVN (Fig. 5A, B), and SON, including the retrochiasmatic part. Moderate clusters of AVP-ir cells were found in the Pe and MPOA, mostly in the MPOAp (Fig. 5C,

D). Scattered AVP-ir cells were found in the AH (Fig. 5E, F), LH (Fig. 5G, H), Rt, SI, and SM. No AVP-ir cells were found in the BST or MeA.

Species differences were found in the density of AVP-ir cells in selected brain areas. Brandt's voles had a higher density of AVP-ir cells in the LH ($F_{(1,28)}=10.1$, $P<0.01$) and Rt ($F_{(1,28)}=11.4$, $P<0.01$) than greater long-tailed hamsters, whereas the latter had a higher density of AVP-ir cells in the MPOA ($F_{(1,28)}=13.2$, $P<0.01$) than the former (Fig. 6). No species or sex differences were found in the density of AVP-ir cells in any other brain areas, and no species–sex interaction was found in any brain areas examined (Table 3).

Scattered AVP-ir fibers were found in several hypothalamic brain areas including the MPOA (Fig. 4C, D), AH, LH, Pe and BST. Dense AVP-ir fibers were found in the PVN and SON (Fig. 4O, P), from which most fibers traveled through the SOX (Fig. 4S, T) to the median eminence. In addition, thalamic AVP-ir fibers were evident in the Rt (Fig. 4G, H) and SI (Fig. 4K, L), and scattered fibers were also seen in the lateral habenular nucleus. Using semi-quantitative measurement, Brandt's voles seemed to have a lower density of AVP-ir fibers in the MPOA than greater long-tailed hamsters (Table 4).

DISCUSSION

Social Brandt's voles and solitary greater long-tailed hamsters show remarkable differences in their social behaviors including reproductive and agonistic behaviors (Yin and Fang, 1998; Zhang et al., 1999, 2001b; Chen and Shi, 2003). In the present study, we examined central OT-ir and AVP-ir staining in the brains of males and females of both species. While OT-ir and AVP-ir stained cells and fibers were found in many brain areas in both species, as reported in other rodents (Sofroniew et al., 1979; DeVries et al., 1985; Rosen et al., 2008), our data indicate species-specific patterns of OT-ir and AVP-ir staining in some brain areas important for social behaviors. These data support our hypothesis that species differences in OT and AVP systems in the brain contribute to species-specific social behaviors. We also found that females had a higher density of OT-ir cells in the PVN than males and that this pattern is similar in both species. However, no other sex differences were found in OT-ir and AVP-ir staining in any other brain areas examined. These data failed to support our prediction that sexually dimorphic patterns of OT and AVP are more apparent in Brandt's voles than in greater long-tailed hamsters.

Species differences in OT and AVP systems

Species differences in OT-ir cells were found in multiple brain regions. Brandt's voles had higher densities of OT-ir cells in the MPOA and MeA than greater long-tailed hamsters, whereas the latter had higher densities of OT-ir cells in the AH and LH than the former. In previous studies in voles, although OT-ir cells were found in the MPOA and LH, no clear differences were detected between vole species with different life strategies (Wang et al., 1996). These voles also did not show apparent species differences in OT receptor binding (Shapiro and Insel, 1992). Furthermore, in a recent study, OT receptor binding in the MPOA did not differ between solitary and social species of tuco-tuco (Beery et al., 2008). Therefore, species differences in the OT system in the present study may be related to species-specific behaviors, rather than general differences in life strategies. For example, central OT has been implicated in a variety of social behaviors and cognitive functions including maternal care, social affiliation, individual recognition, learning and memory (Lim and Young, 2006; Lee et al., 2009). In particular, OT in the MPOA is involved in mating behavior (Caldwell et al., 1989; Caldwell and Moe, 1999), maternal care (Pedersen et al., 1994; Rosen et al., 2008), and social recognition (Popik and van Ree, 1991; Popik et al., 1992), and OT in the MeA plays an important role in social recognition and memory (Ferguson et al., 2001; Choleris et al., 2007). Therefore, high levels of OT in the MPOA and

MeA may be important for the high levels of social behaviors, such as mating and parental care, displayed by social Brandt's voles, compared to greater long-tailed hamsters.

Subregional differences were also found within the MPOA. Brandt's voles had higher densities of OT-ir cells in the anterior and intermediate MPOA than greater long-tailed hamsters whereas the opposite pattern was found in the posterior MPOA. Although the functional significance of such species differences is still unknown, these data suggest that the MPOA is a complex brain structure wherein subnuclei and associated OT systems may have different functions.

Greater long-tailed hamsters showed a higher density of OT-ir cells in the PVN than Brandt's voles. Although not measured, high levels of OT in the PVN may represent higher levels of circulating OT in greater long-tailed hamsters than in Brandt's voles. Seasonal changes in circulating levels of OT and OT receptors in the brain have been found in other animals (Freeman and Currie, 1985; Parker et al., 2001). As our animals were housed under a summer photoperiod, increased OT may make the solitary greater long-tailed hamsters less aggressive and more engaged in social behaviors such as mating, affiliation, and parenting—behaviors expected during breeding seasons.

Species differences were also found in AVP-ir staining. Greater long-tailed hamsters had higher densities of AVP-ir cells in the MPOA than Brandt's voles, whereas an opposite pattern was found in the density of AVP-ir staining in the LH. Although AVP-ir in the MPOA and LH has not been examined in other comparative studies, species differences in AVP-ir staining have been reported in the BST. However, relative levels of AVP-ir staining in this region are not consistent among species that follow the same life strategies. For example, monogamous prairie voles had more AVP-ir cells in the BST than promiscuous meadow voles (Wang, 1995), whereas the opposite pattern was seen between monogamous California mice and polygamous white-footed mice (Bester-Meredith et al., 1999). There is no ready explanation for such contradictory results. Central AVP may contribute to cognitive and behavioral functions in a brain region- and species-specific manner.

Central AVP has been implicated in social recognition, social bonding, flank marking, and aggression (Dantzer et al., 1988; Ferris and Potegal, 1988; Winslow et al., 1993; Liu et al., 2001). In Syrian hamsters, for example, AVP in the MPOA-AH is important for flank marking as local administration of AVP facilitates and an AVP receptor antagonist inhibits flank marking behavior (Hennessey et al., 1994). Both male and female greater long-tailed hamsters possess a pair of flank glands and display high levels of flank marking behavior (Hennessey et al., 1994; Zhang et al., 2001a; Wang et al., 2006), and this behavior plays an important role in social recognition and territory occupation and defense (Ferris et al., 1987; Albers and Rawls, 1989; Gosling, 1990). It is possible that high levels of AVP in the MPOA are related to the flank marking behavior displayed by greater long-tailed hamsters. In our most recent study, dominant male greater long-tailed hamsters expressed a high level of flank marking behavior and this behavior was associated with an enhanced expression of neuronal activation (indicated by Fos-ir staining) in the MPOA, implicating the role of MPOA in flank marking (Pan et al., in press). To our knowledge, Brandt's voles do not display flank marking behavior.

Central AVP in the MPOA-AH also plays an important role in aggression, which has been demonstrated in a variety of species (Ferris and Potegal, 1988; Ritters and Panksepp, 1997; Gobrogge et al., 2009). Although aggressive behavior has been described in both greater long-tailed hamsters and Brandt's voles, the former displays much higher levels of aggression than the latter. We recently found a significant increase in Fos-ir expression in the MPOA and AH associated with offensive and defensive behaviors, respectively, in

greater long-tailed hamsters, indicating a potential role of the MPOA and AH in aggression (Pan et al., in press). That AH AVP plays an important role in aggression is further supported by recent studies in male prairie voles, in which AVP neurons in the AH were activated (indicated by double labeling of AVP-ir/Fos-ir staining) during aggression, and intra-AH AVP manipulation altered aggressive behavior (Gobrogge et al., 2007, 2009). It will be interesting to test, in further experiments, if AVP in the MPOA and AH regulates aggression in greater long-tailed hamsters. Furthermore, high levels of aggression were associated with increased levels of circulating testosterone in male greater long-tailed hamsters (Zhang et al., 2001b; Wang et al., 2009). As gonadal steroid hormones have been found to regulate AVP expression in the MPOA in other rodent species (Johnson et al., 1995; Young et al., 2000), high levels of testosterone may be responsible for enhanced AVP-ir expression in the MPOA of male hamsters, while estrogen may play a similar role in females (De Vries et al., 1994). Needless to say, this speculation needs to be tested in further studies.

Sexually dimorphic neuropeptide staining

Sex differences have been reported in the mammalian brain for several neurotransmitter systems including OT and AVP (De Vries et al., 1983, 1985; Haussler et al., 1990; Lee et al., 2009). Such sex differences may not only underlie sex-specific behavioral and cognitive functions, but may also enable males and females to display similar behaviors despite their physiological differences (De Vries and Boyle, 1998). In the present study, females had a higher density of OT-ir cells in the PVN than males, and this sexual dimorphism was found in both species. This finding is consistent with that from other rodent species such as mice (Haussler et al., 1990). As OT in the PVN plays an important role in mediating reproduction and stress responses (Argiolas and Melis, 1995; Neumann et al., 2000; Nishitani et al., 2004), high OT in the PVN in females may indicate its role in these sexually dimorphic functions. However, our data do not support the prediction that a sexually dimorphic pattern of neuropeptide staining in the brain is more apparent in Brandt's voles than in greater long-tailed hamsters.

In several rodent species including rats, mice, and voles, males are found to have more AVP-ir cells in the BST and MeA than females (De Vries and Panzica, 2006). In the present study, however, AVP-ir cells were virtually absent in the BST and MeA in both species. This is not surprising given that AVP synthesized in the BST and MeA is usually transported rapidly to terminals and treatment with colchicine is required to visualize AVP-ir cells in those brain areas (van Leeuwen and Caffé, 1983; DeVries et al., 1985). Therefore, we cannot exclude the possibility that a sexually dimorphic AVP pathway exists in the BST and MeA, which could be better examined in further experiments by using *in situ* hybridization for AVP mRNA labeling or by doing AVP immunocytochemistry on colchicine-treated animals.

In a related issue, in rats, AVP producing cells in the BST project into some forebrain areas including the LS in which males have a higher density of AVP-ir fibers than females (De Vries and Panzica, 2006). Released AVP in the LS plays an important role in aggression (Compaan et al., 1993; Everts et al., 1997), individual recognition (Bluthe et al., 1990, 1993), and social bonding (Liu et al., 2001) in males. In our study, AVP-ir fibers were not seen in the LS in either species. It should be noted that, in some rodent species such as Syrian hamsters and California mice, the LS does not contain AVP-ir fibers (Dubois-Dauphin et al., 1990; Bester-Meredith et al., 1999). Thus, one possibility is that, like Syrian hamsters, our subjects do not have AVP-ir projections in the LS. This could be the case especially given that greater long-tailed hamsters are very similar to Syrian hamsters in sociality and behaviors (Johnston, 1985; Zhang et al., 2001b). However, it is unclear why the LS of Brandt's voles also does not contain AVP-ir fibers. Another possibility is that the

antibody used in our study might not be sensitive enough to label AVP fibers in the LS. This, however, is unlikely given the fact that clear and specific labeling of AVP-ir fibers were found in other brain regions in both species.

Several caveats should be mentioned. First, although Brandt's voles and greater long-tailed hamsters both belong to the *Cricetidae* family, they live in distinct regions of the world that differ in ecological conditions. As central OT and AVP systems have been implicated in both social behaviors and physiological functions, species differences in OT and AVP systems may also represent physiological, in addition to behavioral, adaptations to the environment. For example, as the LH has been implicated in water and salt intake and AVP plays an important role in water balance (Weitzman and Kleeman, 1979; da Silva et al., 1995; Nielsen et al., 1995), species differences in LH-AVP may have species-specific physiological functions. Second, our subject's housing conditions reflected their species-specific life strategies. However, we cannot exclude the possibility that differences in the degree of social exposure experienced by the two species, due to their differences in housing conditions, may have affected the observed differences in the neuropeptide staining. Therefore, it will be interesting to examine species differences in OT/AVP expression in response to alterations in the social environment in future studies. Finally, although immunocytochemical staining provides excellent anatomical resolution for OT-ir and AVP-ir mapping in the brain, it is only semi-quantitative and cannot be used to measure changes in synthesis or release. Species differences or the lack thereof in OT-ir or AVP-ir fiber densities could result from species differences in the neuropeptide synthesis, release, and/or transportation from cell bodies to terminals. Therefore, caution is required in the interpretation of data.

CONCLUSION

Social Brandt's voles and solitary greater long-tailed hamsters show differences in their social behaviors (Yin and Fang, 1998; Zhang et al., 1999, 2001b; Chen and Shi, 2003). Here we performed a comparative study for immunoreactive staining of OT and AVP in the brain of both species. Our data illustrated species-specific patterns of OT-ir and AVP-ir staining, supporting the hypothesis that species differences in these central neuropeptide systems may underlie species differences in social behaviors. Further, although a sexually dimorphic OT-ir staining pattern was found in the PVN, this pattern was similar in both species, and thus our data did not support the prediction that a sexually dimorphic neuropeptide staining pattern would be more apparent in Brandt's voles than in greater long-tailed hamsters. In previous studies in voles and mice, species with different life strategies and social behaviors differed not only in OT-ir and AVP-ir staining (Wang, 1995; Bester-Meredith and Marler, 2001, 2003), but also in the distribution pattern and regional quantity of OT and AVP receptors, implicating differences in brain responsiveness to released OT and AVP (Insel and Shapiro, 1992; Insel et al., 1994; Bester-Meredith et al., 1999). Therefore, further analysis should be focused on comparative experiments revealing OT and AVP receptor distributions in the brains of Brandt's voles and greater long-tailed hamsters and on pharmacological studies investigating causal relationships between OT/AVP in specific brain regions and social behaviors.

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Abbreviations

AH	anterior hypothalamus, (anterior part)
AHP	anterior hypothalamus, posterior
BST	bed nucleus of the stria terminalis
BSTL	bed nucleus of the stria terminalis, lateral
BSTMA	bed nucleus of the stria terminalis, anterior, medial
BSTMPL	bed nucleus of the stria terminalis, posterior, lateral, medial
BSTV	bed nucleus of the stria terminalis, ventral
LH	lateral hypothalamic area
LS	lateral septum
MeA	medial amygdaloid nucleus
MnPO	median preoptic nucleus
MPOA	medial preoptic area
MPOAa	medial preoptic area, anterior
MPOAi	medial preoptic area, intermediate
MPOAp	medial preoptic area, posterior
opt	optic tract
Pe	paraventricular hypothalamic nucleus
PVN	paraventricular nucleus of the hypothalamus
Rt	reticular thalamic nucleus
SCN	suprachiasmatic nucleus
SI	substantia innominata
SM	nucleus of stria medullaris
SON	supraoptic nucleus
SOX	supraoptic decussation
VMH	ventromedial hypothalamus
3V	third ventricle

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Fig. 1. Photomicrographs displaying OT-ir stained cells and fibers in the paraventricular nucleus of the hypothalamus (PVN; A, B), anterior hypothalamus (AH; C, D), lateral hypothalamus (LH; E, F), and medial nucleus of the amygdala (MeA; G, H) in the brains of Brandt's voles (A, C, E, G) and greater long-tailed hamsters (B, D, F, H). 3V, third ventricle; opt, optic tract; Scale bar=100 μ m.

Fig. 2. Photomicrographs displaying OT-ir stained cells and fibers in the anterior (MPOAa; A, B), intermediate (MPOAi; C, D), and posterior (MPOAp; E, F) part of the medial preoptic area (MPOA) in the brains of Brandt's voles (A, C, E), and greater long-tailed hamsters (B, D, F). 3V, third ventricle, Scale bar=100 μm.

Fig. 3.

(A) Species differences in the density of OT-ir cells in the reticular thalamic nucleus (Rt), medial preoptic area (MPOA), anterior hypothalamus (AH), lateral hypothalamus (LH), and medial nucleus of the amygdala (MeA). The two species also differed in the density of OT-ir cells in (B) the paraventricular nucleus of the hypothalamus (PVN) and (C) the subnuclei of the MPOA, including the anterior (MPOAa), intermediate (MPOAi), and posterior (MPOAp) part of the MPOA. * $P < 0.05$, ** $P < 0.01$.

Fig. 4. Photomicrographs displaying OT-ir and AVP-ir fibers in the brains of Brandts' voles (BV) and greater long-tailed hamsters (GH). OT-ir fibers were found in the bed nucleus of the stria terminalis, posterolateral-medial part (BSTMPL; A, B), lateral hypothalamus (LH; E, F), substantia innominate (SI; I, J), supraoptic nucleus (SON; M, N), and supraoptic decussation (SOX; Q, R). AVP-ir fibers were found in the medial preoptic area (MPOA; C, D), reticular thalamic nucleus (Rt; G, H), SI (K, L), SON (O, P), and SOX (S, T). Scale bar=100 μ m.

Fig. 5. Photomicrographs displaying AVP-ir stained cells and fibers in the paraventricular nucleus of the hypothalamus (PVN; A, B), medial preoptic area (MPOA; C, D), anterior hypothalamus (AH; E, F), and lateral hypothalamus (LH; G, H) in the brains of Brandt's voles (A, C, E, G) and greater long-tailed hamsters (B, D, F, H). 3V, third ventricle, Scale bar=100 μ m.

Fig. 6.

(A) Species differences in the density of AVP-ir cells in the reticular thalamic nucleus (Rt), medial preoptic area (MPOA), anterior hypothalamus (AH), and lateral hypothalamus (LH). (B) AVP-ir cells were also found in the paraventricular nucleus of the hypothalamus (PVN).

* $P < 0.05$, ** $P < 0.01$.

Density of oxytocin immunoreactive cells (mean±SEM/mm²) in male and female Brandt's voles and greater long-tailed hamsters

Table 1

Brain area	Brandt's vole		Greater long-tailed hamster		Two-way ANOVA		
	Male	Female	Male	Female	Species	Sex	Sp×sex
Rt	12.2±4.3	12.0±1.8	12.7±1.4	7.9±1.2	ns	ns	ns
MPOA	27.7±3.7	38.0±4.7	16.4±1.9	15.5±1.8	**	ns	ns
MPOAa	58.5±10.0	89.4±14.1	14.0±2.2	11.7±1.6	**	ns	ns
MPOAi	14.2±4.2	12.5±1.7	4.1±0.6	3.6±0.4	**	ns	ns
MPOAp	10.5±2.3	11.3±1.3	31.2±4.5	31.1±4.5	**	ns	ns
AH	3.4±1.4	3.1±0.8	6.2±1.0	4.9±0.9	*	ns	ns
AHP ^a	34.3±2.8	38.3±4.2	31.6±3.1	36.2±3.8	ns	ns	ns
LH	3.5±0.8	4.3±1.0	5.8±0.6	5.9±1.0	*	ns	ns
MeA	0.9±0.4	1.3±0.3	0.4±0.1	0.3±0.1	**	ns	ns
PVN	488.4±27.4	613.4±37.6	819.4±32.0	835.1±40.3	**	*	ns

SEM, standard error of the mean;

* $P<0.05$;

** $P<0.01$;

ns, not significantly different.

^a absolute cell numbers because the boundary of the brain area was obscured in nissl staining.

OT-ir fiber density in the brain of Brandt's voles (BV) and greater long-tailed hamsters (GH)

Table 2

	Rt	MPOA	AH	LH	PVN	Pe	Si	SON	SOX	BSTMPL
BV	++/++++	++	++/++++	++	+++	+++	+/++	+++	+++	++
GH	++/++++	+/+	++/++++	+/++	+++	+++	+/++	+++	+++	++

Density of vasopressin immunoreactive cells (mean±SEM/mm²) in male and female Brandt's voles and greater long-tailed hamsters

Table 3

Brain area	Brandt's vole		Greater long-tailed hamster		Two-way ANOVA		
	Male	Female	Male	Female	Species	Sex	Sp×sex
Rt	35.2±6.9	22.9±4.8	12.9±3.4	14.0±2.1	**	ns	ns
MPOA	17.6±1.7	17.3±2.3	27.0±3.8	28.7±3.2	**	ns	ns
AH	13.1 ±2.0	13.3±3.4	13.2 ±1.9	10.9± 1.1	ns	ns	ns
LH	15.6±1.2	13.2±1.4	10.9±0.5	10.8±1.2	**	ns	ns
PVN	596.8±19.9	700.4±43.9	647.2±25.3	636.2±23.7	ns	ns	ns

SEM, standard error of the mean;

**
P<0.01;

ns, not significantly different.

AVP-ir fiber density in the brain of Brandt's voles (BV) and greater long-tailed hamsters (GH)

Table 4

	Rt	MPOA	AH	LH	PVN	Pe	Si	SON	SOX
BV	++	+	+ +/+ + + +	++	+ +/+ + + +	+++	++	+++	+++
GH	++	+ /++	+ +/+ + + +	++	+ +/+ + + +	+++	+ /++	+++	+++