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# Anterior Hypothalamic Neural Activation and Neurochemical Associations with Aggression in Pair-Bonded Male Prairie Voles

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ABSTRACT

Male prairie voles (*Microtus ochrogaster*) display mating-induced pair bonding indicated by social affiliation with their female partners and aggression toward unfamiliar conspecifics. In the present study, we characterized their aggression associated with pair bonding and examined the related neuronal activation and neurochemical architecture. Males that were pair-bonded for 2 weeks displayed intense levels of aggression toward a female or male conspecific stranger but maintained a high level of social affiliation with their familiar female partners. These social interactions induced increases in neural activation, indicated by increased density of Fos-immunoreactive staining (Fos-ir) in several brain regions including the bed nucleus of the stria terminalis (BNST), medial preoptic area (MPOA), paraventricular nucleus (PVN), anterior cortical (AcA), and medial nuclei (MeA) of the amygdala. In the anterior hypothalamus (AH), increased density of Fos-ir staining was found specifically to be associated with aggression toward unfamiliar female or male strangers. In addition, higher densities of AH cells that were stained for tyrosine hydroxylase (TH) or vasopressin (AVP) were also labeled with Fos-ir in these males displaying aggression toward a conspecific stranger compared with males displaying social affiliation toward their female partner. Together, our results indicate that dopamine and vasopressin in the AH may be involved in the regulation of enduring aggression associated with pair bonding in male prairie voles. J. Comp. Neurol. 502:1109–1122, 2007. 

**Indexing terms: dopamine; vasopressin; anterior hypothalamus; neural activation; *c-fos*; mating; aggression**

Several animal models have been used to uncover the neurochemical and behavioral profile of aggression (Adams, 2006; Ramirez, 2006). In most studies, animals were switched from nonaggressive to aggressive by manipulations of their physiological conditions (Ferris et al., 1997; DeLeon et al., 2002b; Miczek et al., 2004; Nelson, 2005; Wommack et al., 2005). Within these studies, typically only one form of social behavior, aggression, was investigated. Little attention has been paid to other forms of social behaviors including "general" social olfactory processing or other forms of prosocial behaviors such as affiliation. Given that considerable overlap of brain areas is involved in several forms of social behaviors (Newman, 1999), aggression being only one of them, there is a significant amount of ambiguity regarding which brain areas may be involved in the regulation of aggression.

In the natural environment, many animals can display high levels of both social affiliation and aggression, but they direct these behaviors differentially toward conspecifics. This differential pattern of behavior is dependent on the animals' previous social and sexual experience and/or subsequent exposure to various social stimuli (Wang et al., 1997). Unfortunately, due to the lack of appropriate animal models, little is known about the neurochemical

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transformations that take place when an animal switches from expressing one type of behavior, such as social affiliation, to another, such as aggression (McNamara et al., 2005). Nonetheless, the results from early studies indicate the involvement of brain structures, such as the anterior hypothalamus (AH; Ferris and Potegal, 1988; Adams et al., 1993; Delville et al., 2000; DeLeon et al., 2002b; Albers et al., 2006) and lateral septum (Albert and Brayley, 1979; David et al., 2004; Knyshevski et al., 2005a; Trainor et al., 2006), and neurochemicals, such as vasopressin (AVP; Ferris et al., 1997), dopamine (DA; Ago and Matsuda, 2003; Ferrari et al., 2003), oxytocin (OXT; Ebner et al., 2005), and serotonin (5-HT; Ago and Matsuda, 2003; Ferrari et al., 2003; Ferris et al., 1997), in the regulation of the onset of aggressive behavior.

The prairie vole (*Microtus ochrogaster*) is a monogamous rodent species that displays mating-induced pair bonding. In the lab, pair bonding is indicated by social affiliation, preferentially with a familiar mate versus a conspecific stranger (partner preference) and "intense" aggression toward a stranger (selective aggression). Although extended cohabitation can induce pair bonding in female prairie voles (Williams et al., 1992b), mating seems to be essential for pair bond formation in males (Winslow et al., 1993; Insel et al., 1995). It is important to note that a pair bond, once formed, is enduring: more than 90% of prairie voles did not form new pairs after the original partners died or were removed in the field (Getz et al., 1981), and pair bonding lasts for at least 2 weeks even in the absence of continuing exposure to a partner in the lab (Insel et al., 1995; Aragona et al., 2006). It has been suggested that selective aggression is a behavioral trait associated with mate guarding and thus plays an important role in the maintenance of monogamous pair bonding (Carter, 2003; Aragona et al., 2006).

Prairie voles have been used as an excellent animal model for study of the neuroanatomical and neurochemical regulation of adult social attachment (Curtis et al., 2006; Wang and Aragona, 2004; Young and Wang, 2004), which has direct implications for better understanding of the neurobiology of human disorders characterized by social deficits, such as autism (Lim and Young, 2006). Most studies have focused on characterizing partner preference formation, and several neurotransmitter systems, including AVP, OXT, DA, GABA, and glutamate, have been implicated in partner preference formation in male and female prairie voles (Williams et al., 1992a; Winslow et al., 1993; Carter et al., 1995; Aragona et al., 2003; Liu and Wang, 2003; Lim et al., 2004a; Curtis and Wang, 2005b). Some attempts have also been made to study the neurobiology of selective aggression in male prairie voles. It has been found that selective aggression is induced by 24 hours of mating (Winslow et al., 1993; Insel et al., 1995; Wang et al., 1997). This behavior is associated with specific patterns of neuronal activation in the brain (Wang et al., 1997) and can be blocked by central infusion of the AVP V1a receptor antagonist (Winslow et al., 1993). In our most recent study, central administration of the DA D1-type receptor antagonist diminished selective aggression in pair-bonded male prairie voles, implicating involvement of central DA in this behavior (Aragona et al., 2006).

Although these previous studies indicate that selective aggression is an enduring characteristic behavior associated with a monogamous life strategy and is regulated by central neurotransmitter systems including AVP and DA, our understanding of the neurobiology of selective aggression is still limited. For example, it is unknown whether selective aggression, after 2 weeks of pair bonding, is regulated by the same or different neural circuits compared with the aggression following 24 hours of mating (Wang et al., 1997). Furthermore, it is unknown whether different neurochemicals (such as AVP and DA) are involved in different stages of selective aggression. In the present study, we first tested the behaviors of male prairie voles that were pair-bonded for 2 weeks to further characterize their selective aggression toward conspecific intruders. We also used a protein product (Fos) of an immediate-early gene, *c-fos*, to reveal the neuronal activation associated with selective aggression. Finally, we focused on the anterior hypothalamus, a brain area that

*Abbreviations*

| | |
|---|---|
| 3V | third ventricle |
| 5-HT | serotonin |
| AcA | anterior cortical amygdala |
| Aco | anterior cortical amygdaloid nucleus |
| AH | anterior hypothalamus |
| AHA | anterior hypothalamus, anterior |
| AHC | anterior hypothalamus, central |
| AHP | anterior hypothalamus, posterior |
| AVP | vasopressin |
| BNST | bed nucleus of the stria terminalis |
| BSTMA | bed of the stria terminalis, medial division, anterior |
| cAMP | cyclic adenosine monophosphate |
| DA | dopamine |
| EB | estradiol benzoate |
| F | fornix |
| GABA | γ-aminobutyric acid |
| ir | immunoreactive, immunoreactivity |
| K-W | Kruskal-Wallis |
| LS | lateral septum |
| LSI | lateral septal nucleus, intermediate |
| LSV | lateral septal nucleus, ventral part |
| LV | lateral ventricle |
| MeA | medial amygdala |
| MeAD | medial amygdala nucleus, anterodorsal |
| MeAV | medial amygdala nucleus, anteroventral |
| MePV | medial amygdala nucleus, posteroventral |
| MePD | medial amygdala nucleus, posterodorsal |
| MM | medial mammillary nucleus |
| MPA | medial preoptic area |
| MPOA | medial preoptic area |
| MT | medial terminal optic tract |
| NAcc | nucleus accumbens |
| NC | nucleus circularis |
| OT | optic tract |
| OXT | oxytocin |
| Pa | paraventricular hypothalamic nucleus |
| PaAP | Pa, anterior parvicellular part |
| PaDC | Pa, dorsal cap |
| PaLM | Pa, lateral magnocellular |
| PaMP | Pa, medial parvicellular |
| PaV | Pa, ventral part |
| Pe | periventricular hypothalamic nucleus |
| PVN | paraventricular nucleus |
| SN | substantia nigra |
| SNK | Student-Newman-Keuls |
| SON | supraoptic nucleus |
| TH | tyrosine hydroxylase |
| VTA | ventral tegmental area |

has been implicated in aggression in other rodent and mammalian species (Olivier et al., 1983; Fuchs et al., 1985; Ferris et al., 1997; Delville et al., 2000; Jackson et al., 2005; Albers et al., 2006; Trainor et al., 2006). In the present study, the AH was activated by selective aggression in male prairie voles, therefore we examined the neurochemical phenotypes of these Fos-immunoreactive (Fos-ir) labeled cells. Together, our neuroanatomical and neurochemical data illustrate that the AH may be a brain area in which AVP and DA exert their functions in the regulation of aggression associated with pair bonding in male prairie voles.

## MATERIALS AND METHODS

### Subjects

Subjects were sexually naive adult male prairie voles (*Microtus ochrogaster*) that were offspring of the F4 generation of a laboratory breeding colony. The voles were weaned at 21 days of age and housed in same-sex sibling pairs in plastic cages (29 × 18 × 13 cm) containing cedar chip bedding. All cages were maintained under a light-dark photoperiod of 14:10 hours photoperiod with lights on at 0700 hours. Temperature was maintained at 21 ± 1°C. Animals were provided ad libitum food (Purina Hi-Fiber Rabbit Chow 5326 and black oil sunflower seeds) and water.

### Mating and social cohabitation paradigm

At 3–5 months of age, male subjects were paired with a sexually receptive female for 24 hours, and their behavior was videotaped to ensure mating. The females were estrogen primed with one daily injection of estradiol benzoate (EB) at 1 μg/100 μl sesame oil for 4 days, which successfully induced behavioral estrus (Fowler et al., 2005). Thereafter, pairs were housed together in plastic cages (29 × 18 × 13 cm). On day 12 after pairing, pairs were transferred into larger cages (45 × 22 × 20 cm) with their original bedding to allow for more movement during the resident intruder test (see below). On the morning of the 15th day, male subjects were acclimated in their home cage for 5 minutes after their female partners were removed and then were randomly assigned to one of three social groups for resident intruder testing; 1) re-exposed to their familiar female partner, 2) exposed to an unfamiliar female, or 3) exposed to an unfamiliar male (both intruders age and size matched). The unfamiliar females in group 2 were ovariectomized 2 weeks prior to testing and then given 4-day EB priming prior to the resident intruder test. Finally, a fourth group of males was not exposed to any type of intruder after their female partners were removed, and this group served as the baseline control for the immunocytochemistry experiments (see below).

### Resident intruder testing

Behavioral interactions between the male subject and intruder in the first three groups were videotaped for 10 minutes. The following behavioral patterns of the resident were subsequently quantified: the frequency of attacks, bites, chases, defensive/offensive upright postures, offensive sniffs, threats, and retaliatory attacks (directly following an intruder attack) as well as the duration of affiliative side-by-side contact (Winslow et al., 1993) and time spent during anogenital investigation. After behavioral testing, intruders were immediately removed, and male residents were housed alone for 2 hours before perfusion. Female partners were sacrificed and dissected to ensure pregnancy. All females were pregnant. Animal procedures were approved by Florida State University's Animal Care and Use Committee and conformed to NIH guidelines.

### Tissue preparation

Subjects were anesthetized with sodium pentobarbital (0.1 mg/10 g body weight) and then perfused through the ascending aorta with 100 ml 0.9% saline, followed by 100 ml 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4). Brains were harvested, postfixed for 2 hours in 4% paraformaldehyde, and then stored in 30% sucrose in PBS. Next, brains were cut into 30-μm coronal sections on a freezing microtome, and the floating sections were stored in 0.1 M PBS with 1% sodium azide until processing for Fos, tyrosine hydroxylase (TH), and AVP immunocytochemistry.

### Fos, TH, and AVP immunocytochemistry

Floating brain sections at 180-μm intervals were processed for Fos/TH double-label immunostaining. Sections were rinsed in 0.05 M Tris-NaCl (pH 7.6) for 30 minutes and then with 0.5% $NaBH_4$ in 0.05 M Tris-NaCl at room temperature for 10 minutes. After rinsing in 0.05 M Tris-NaCl for 25 minutes, sections were incubated in 0.006% hydrogen peroxide in 0.05 M Tris-NaCl for 30 minutes; rinsed in 0.05 M Tris-NaCl for 15 minutes; and incubated in 0.3% Triton X-100 in 0.05 M Tris-NaCl with 10% normal goat serum for 1 hour and in rabbit anti-Fos polycolonal IgG (c-Fos [4]-G: *sc*-52; 1:20,000; Santa Cruz Biotechnology, Santa Cruz, CA, lot no. C1805) in 0.3% Triton X-100 in 0.05 M Tris-NaCl with 2% normal goat serum at 4°C overnight. Thereafter, sections were continuously incubated in rabbit anti-Fos IgG at room temperature for 2 hours; rinsed in 0.3% Triton X-100 in 0.05 M Tris-NaCl for 30 minutes; and incubated in biotinylated goat anti-rabbit IgG (BA-1000; 1:300; Jackson ImmunoResearch, West Grove, PA, lot no. Q0611) in 0.3% Triton X-100 in 0.05 M Tris-NaCl with 2% normal goat serum for 2 hours. Sections were rinsed in 0.3% Triton X-100 in 0.05 M Tris-NaCl for 15 minutes; then rinsed in 0.05 M Tris-NaCl for 15 minutes; incubated in avidin-biotin complex (Vectastain Elite, Vector, Burlingame, CA) in 0.05 M Tris-NaCl for 90 minutes; and rinsed in 0.05 M Tris-NaCl for 25 minutes. Nuclear labeling was immunoreactively stained by using 3′-diaminobenzidine (1 DAB/$H_2O_2$ tablet; Sigma, St. Louis, MO) and 3 mg NiCl powder in 5 ml deonized/distilled $H_2O$ for 8 minutes, revealing black punctate nuclear staining. Sections were then rinsed in 0.3% Triton X-100 in 0.05 M Tris-NaCl for 2 minutes to stop the reaction and then rinsed in 0.05 M Tris-NaCl for 30 minutes.

Next, sections were incubated in 0.2% Triton X-100 in 0.05 M Tris-NaCl with 5% normal goat serum for 30 minutes and then incubated in rabbit anti-TH (AB152; 1:7,000; Chemicon, Temecula, CA; lot no. 25020194) in 0.2% Triton X-100 in 0.05 M Tris-NaCl with 2% normal goat serum overnight. Sections were then incubated at room temperature for 2 hours; rinsed in 0.3% Triton X-100 in 0.05 M Tris-NaCl with 2% normal goat serum for 30 minutes; and incubated in biotinylated goat anti-rabbit IgG (BA-1000; 1:300; Jackson ImmunoResearch; lot no.

Q0611) in 0.3% Triton X-100 in 0.05 M Tris-NaCl with 2% normal goat serum for 2 hours at room temperature. Sections were rinsed in 0.3% Triton X-100 in 0.05 M Tris-NaCl for 15 minutes; rinsed in 0.05 M Tris-NaCl for 15 minutes; incubated in avidin-biotin complex (Vector) in 0.05 M Tris-NaCl for 90 minutes; and rinsed in 0.05 M Tris-NaCl for 25 minutes. Cytoplasmic labeling was immunoreactively stained for by using a Sigma DAB Kit (2 drops DAB and 2 drops Buffer) in 5 ml deonized/distilled $H_2O$ for 5 minutes and then reacted with 2 drops $H_2O_2$ for 3 minutes, revealing brown cytoplasmic staining. Sections were rinsed in 0.3% Triton X-100 in 0.05 M Tris-NaCl for 2 minutes to stop the reaction and then rinsed in 0.05 M Tris-NaCl for 30 minutes.

Because Fos-ir and TH-ir/Fos-ir data illustrated that activation of the AH was associated with selective aggression toward strangers and AVP in this area is implicated in aggression in other rodent species (Ferris and Potegal, 1988; Jackson et al., 2005; Albers et al., 2006), another set of brain sections at 180-μm intervals was processed for Fos/AVP double immunocytochemistry. The same immunocytochemistry protocol outlined above was used except that the primary antibody for the AVP labeling was a rabbit polyclonal anti-AVP (cat no. 64717; 1:15,000; ICN Biomedicals, Aurora, OH; lot no. 1143C).

## Specificity of antisera

All three primary antibodies have been previously characterized and used in vole brain tissue. As communicated by the manufacturer, the anti-c-Fos antibody was raised against the N-terminal sequence (residues 1–16) of c-Fos protein that is conserved among mouse, rat, and human. This antibody recognized a ~62 kDa c-Fos on Western blot, which corresponds to the expected molecular weight of c-Fos (manufacturer's technical information). In our previous studies in voles, overnight absorption of the Fos antiserum with 10-fold of the control peptide (Santa Cruz Biotechnology) completely eliminated specific labeling (Wang et al., 1997).

The immunogen for the TH primary antibody is denatured TH from rat pheochromocytoma, which has been shown by Western blotting to have a molecular weight of ~62 kDa and not to recognize other monoaminergic synthetic enzymes (manufacturer's technical information). Omission of the primary antibody abolished any specific labeling. In addition, previous reports in prairie voles using this antibody have shown TH labeling in brain areas described to express TH in other rodent species (Lansing and Lonstein, 2006; Northcutt et al., 2007).

The rabbit polyclonal anti-AVP was raised against synthetic AVP (full length) linked to the carrier protein thyroglobulin (manufacturer's technical information). In previous studies in voles, omission of this antibody or concurrent incubation of this antibody with 50 μM AVP eliminated specific staining (Wang et al., 1996), and this antibody did not cross-react with 50 μM oxytocin (Bamshad et al., 1993).

For each of the double-labeling experiments (TH-ir/Fos-ir and AVP-ir/Fos-ir), we used a two-step immunocytochemistry protocol, first by using DAB with NiCl to reveal black nuclear staining for Fos and then by using DAB only to reveal brown cytoplasmic staining for TH or AVP. This sequential staining procedure led to saturation of all binding sites for Fos-ir nuclear staining, preventing cross-reactions when the second cytoplasmic staining was conducted. The specificity of the double labeling was also evidenced by the presence of single-labeled cells for TH, AVP, and Fos. In addition, in a recent pilot experiment, we performed triple immunofluorescence labeling for Fos (rabbit-anti-Fos polycolonal IgG; c-Fos [4]-G: *sc*-52; 1:20,000; Santa Cruz Biotechnology; lot no. C1805), TH (mouse anti-TH monoclonal IgG; MAB318, 1:2,000, Chemicon; lot no. 0505000106), and AVP (guinea pig anti-AVP monoclonal IgG, 1:3,200; Peninsula Lab, San Carlos, CA; lot no. 030337) on several remaining brain sections from the above experiment. Stereological microscopy confirmed similar patterns of the single and double (and some triple) labeled cells in the AH.

## Data quantification and analysis

The frequency and duration of behaviors during the resident intruder test were quantified by a research assistant, blind to group identification and study conditions, and analyzed by a Kruskal-Wallis (K-W) nonparametric test (because of nonhomogeneity of variance), followed by K-W testing of each possible pair of means by using a Bonferroni correction for the number of comparisons. In addition, several behaviors (including attack, bite, chase, threat, offensive/defensive upright, offensive sniff, and resident retaliatory attack) were grouped as a composite aggression score and then similarly analyzed.

Previous studies examining neuronal activation in voles have relied on profile cell counting methods, which now are considered to be less sophisticated than stereological counting methods (Mura et al., 2004). Therefore, stereological quantification was used in the present study. Group identification was blind to the research assistant analyzing slides. Fos-ir or TH-ir single-labeled cells and cells labeled for both TH-ir/Fos-ir were identified and quantified bilaterally in the following brain regions: the lateral septum (LS), bed nucleus of the stria terminalis (BNST)—particularly the anterior medial division of the BST, medial preoptic area (MPOA), paraventricular nucleus (PVN), anterior hypothalamus (AH), medial (MeA) and anterior cortical (AcA) amygdala, and ventral tegmental area (VTA). In addition, AVP-ir single-labeled and AVP-ir/Fos-ir double-labeled cells were identified and quantified bilaterally in the AH. Brain regions were defined by using the Paxinos and Watson (1998) rat brain atlas. All brain areas were quantified under 40× magnification by using the optical fractionator probe with Stereo Investigator computer software (MicroBrightField, Colchester, VT) on a Leica DMRB microscope. The stereological parameters, anatomical coordinates (i.e., Paxinos and Watson, 1998, plates), and subnuclei abbreviations for each brain area quantified are summarized in Table 1.

Each brain area was completely traced at 5× magnification and randomly sampled with a preprogrammed sampling grid and counting frame size (Table 1). Nonbiased cell counts were performed by using Stereo Investigator software. Sections that were missing and/or damaged, across all brain areas examined, were not included in the stereological traces; however this rarely occurred due to the large area that each brain nucleus spanned throughout the available sections stained. The average mounted section thickness was approximately 10 μm after processing; therefore the optical dissector height was set to 6 μm prior to each measurement. The average Gundersen coefficient of error was less than 0.2 for each cell count across all areas examined. Cell density (number of cells

TABLE 1. Stereology Quantification Parameters[1]

| Brain area | No. of sections | Sampling grid size (μm) | Counting frame size (μm) | No. of sampling sites | Paxinos and Watson plate no.[2] | Paxinos and Watson anatomical abbreviations |
|---|---|---|---|---|---|---|
| LS | 3 | 250 × 250 | 100 × 100 | 100 | 18–20 | LSI, LSV |
| BNST | 3 | 100 × 150 | 75 × 100 | 100 | 18–20 | BSTMA |
| MPOA | 3 | 250 × 250 | 100 × 100 | 100 | 19–22 | MPA |
| PVN | 8 | 50 × 50 | 50 × 87.5 | 50 | 24–27 | Pe, PaAP, PaMP, PaV, PaLM, PaDC |
| AH | 3 | 150 × 200 | 100 × 100 | 100 | 24–27 | AHA, AHC, AHP |
| MeA | 6 | 100 × 100 | 100 × 100 | 40 | 26–34 | MeAD, MeAV, MePV, MePD |
| AcA | 3 | 100 × 200 | 100 × 100 | 30 | 26–31 | ACo |
| VTA | 3 | 250 × 250 | 100 × 100 | 100 | 40–44 | VTA |

[1]Each brain area was measured through the entire rostral to caudal extent of the available sections at 180-μm intervals. For abbreviations, see list.
[2]Paxinos and Watson, 1998.

TABLE 2. Behavioral Patterns During Resident Intruder Testing[1]

| Behavior | Partner (n = 9) | Female intruder (n = 10) | Male intruder (n = 12) | H[2] | P |
|---|---|---|---|---|---|
| Resident behavior frequency | | | | | |
| Attack | $2.44 \pm 2.20^{\alpha}$ | $10.40 \pm 4.84^{\alpha}$ | $37.83 \pm 7.17^{\beta}$ | 16.77 | <0.001 |
| Bite | $0.56 \pm 0.56^{\alpha}$ | $2.70 \pm 1.61^{\alpha}$ | $21.00 \pm 5.48^{\beta}$ | 15.33 | <0.001 |
| Chase | —$^{\alpha}$ | $12.50 \pm 4.63^{\beta}$ | $21.58 \pm 3.05^{\beta}$ | 19.83 | <0.001 |
| Defensive upright | —$^{\alpha}$ | $8.80 \pm 1.96^{\beta}$ | $7.83 \pm 1.97^{\beta}$ | 16.38 | <0.001 |
| Offensive sniff | —$^{\alpha}$ | $3.00 \pm 1.02^{\beta}$ | $2.33 \pm 0.88^{\beta}$ | 10.28 | <0.01 |
| Offensive upright | $0.22 \pm 0.22^{\alpha}$ | $4.70 \pm 1.48^{\beta}$ | $8.33 \pm 1.53^{\beta}$ | 16.41 | <0.001 |
| Resident retaliatory attack | —$^{\alpha}$ | $3.90 \pm 1.83^{\beta}$ | $2.33 \pm 0.97^{\beta}$ | 10.29 | <0.01 |
| Threat | $0.11 \pm 0.11^{\alpha}$ | $0.90 \pm 0.69^{\alpha,\beta}$ | $1.83 \pm 0.53^{\beta}$ | 9.40 | <0.01 |
| Resident behavior duration (sec) | | | | | |
| Anogenital investigation | $97.78 \pm 14.14^{\alpha}$ | $16.80 \pm 2.87^{\beta}$ | $48.33 \pm 11.01^{\alpha}$ | 14.11 | <0.001 |

[1]Data are presented as mean ± standard error. Greek letters indicate results of the Kruskal-Wallis test with Bonferroni correction. Groups with different letters differ significantly from each other.
[2]Kruskal-Wallis one-way analysis of variance test statistic.

per $mm^3$, for both single and double labeling) of representative sections throughout each brain area was quantified and calculated for each animal. Group differences in the density of labeled cells for each brain area were analyzed by a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls (SNK) post hoc test. Photomicrographs were captured by using a Zeiss Axioskop 2 (Carl Zeiss) microscope with a SPOT RT Slider (Diagnostic Instruments) camera and SPOT™ (version 3.0.6) software. They were then stored and minimally processed for contrast and brightness by using Adobe Photoshop (version 8.0; Adobe Systems, San Jose, CA) software.

# RESULTS

## Selective aggression and affiliative behavior

Male prairie voles that were pair-bonded for 2 weeks displayed different behavioral responses toward different types of conspecific intruders (Table 2). When several behavioral patterns (including attack, bite, chase, threat, offensive and defensive upright posturing, offensive sniff, and retaliatory attack) were grouped as a total score for aggressive behavior, clear group differences emerged ($H_{(2,28)} = 19.61$, $P < 0.001$; Fig. 1A). The K-W test indicated that these males displayed significantly higher levels of aggression toward an unfamiliar female intruder than toward the familiar female partner, and this level of aggression was further elevated when the intruder was an unfamiliar male. Group differences were also found for affiliative behavior ($H_{(2,28)} = 19.17$, $P < 0.001$; Fig. 1B). The K-W test indicated that these males displayed significantly higher levels of affiliative side-by-side contact with their familiar partners in comparison with an unfamiliar female or male intruder.

## Neural activation associated with resident intruder testing

Fos immunocytochemistry resulted in dense nuclear staining in all brain regions examined in the present experiment (see Figs. 3, 4, 6). A resident intruder test induced increases in the density of Fos-ir stained cells in many brain regions in a stimulus- and region-specific manner. Among the brain regions examined, three types of Fos-ir activation were found. First, group differences in the density of Fos-ir stained cells were found in the BNST ($F_{(3,35)} = 5.62$, $P < 0.01$; Fig. 2B), MPOA ($F_{(3,35)} = 7.64$, $P < 0.001$; Fig. 2C), PVN ($F_{(3,35)} = 4.75$, $P < 0.01$; Fig. 2D), AcA ($F_{(3,35)} = 5.15$, $P < 0.01$; Figs. 2G, 3I–L) and MeA ($F_{(3,35)} = 5.77$, $P < 0.01$; Figs. 2F, 3I–L). The SNK post hoc test indicated that after the 10-minute resident-intruder test, male subjects had a higher density of Fos-ir cells in those brain regions in comparison with the baseline control group, indicating that increased Fos-ir expression was associated with behavioral testing. However, no significant differences were found in the density of Fos-ir cells among the groups that experienced resident intruder testing. Second, a group difference in the density of Fos-ir cells was also found in the AH ($F_{(3,35)} = 14.13$, $P < 0.001$, Figs. 2E, 3E–H). The SNK post hoc test indicated that males displayed an increase in the density of Fos-ir cells after being re-exposed to their familiar partner, in comparison with the baseline controls. However, exposure to a female or a male stranger resulted in further significant increases in the density of Fos-ir cells in the AH. Finally, no group differences were found in the density of Fos-ir labeling in the LS ($F_{(3,35)} = 1.89$, $P = 0.16$; Figs. 2A, 3A–D) and VTA ($F_{(3,35)} = 1.1$, $P = 0.37$; Fig. 2H).

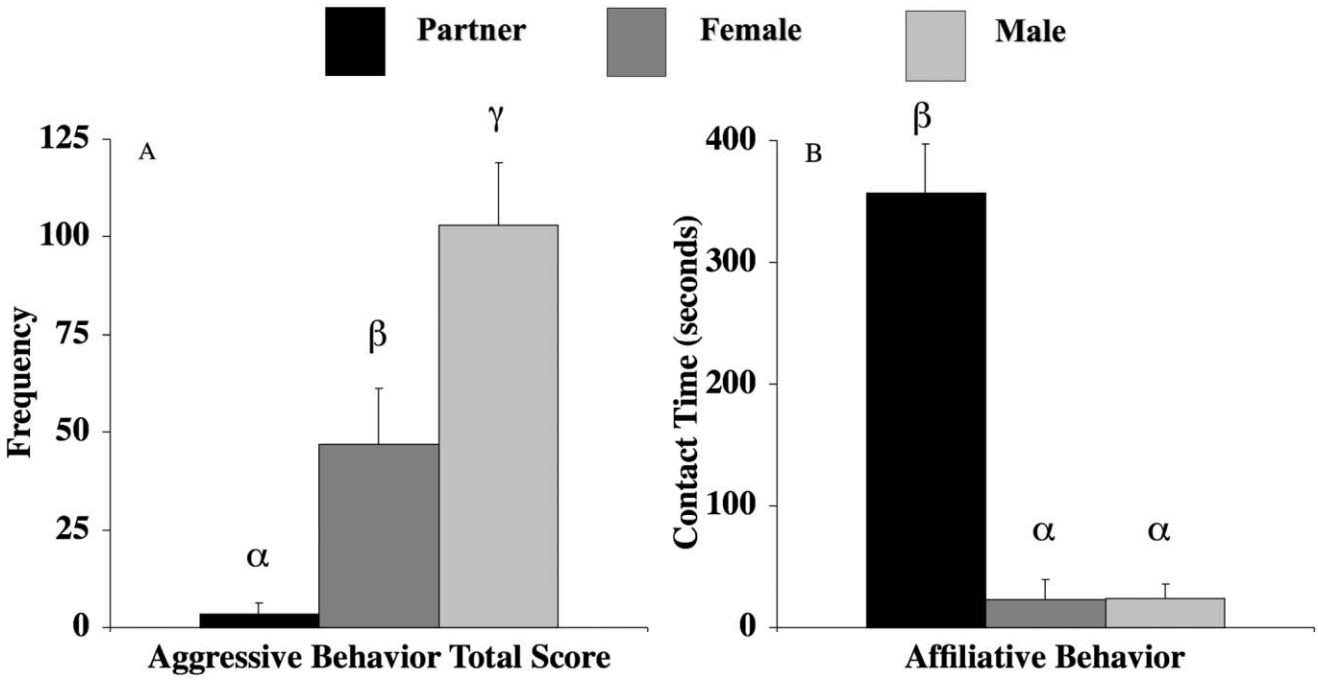


Fig. 1. Mating induces selective aggression and affiliation in pair-bonded male prairie voles. Male prairie voles that were pair-bonded for 2 weeks displayed high levels of aggressive behavior toward unfamiliar male and female intruders (**A**) and a high level of affiliation toward their familiar female partner (**B**). Bars with different Greek letters differ significantly from each other. Error bars indicate standard error of the mean.

## TH-ir positive cells associated with resident intruder testing

TH-ir stained cells were found in all brain regions examined. Specifically, TH-ir cells were found in the intermediate lateral septal nucleus (LSI) of the LS, bed nucleus of the stria terminalis anterior medial division (BSTMA), medial preoptic nucleus (MPA), throughout all subdivisions quantified in the PVN, anterior hypothalamic area (AH) ventral to the fornix, medial supraoptic nucleus (SON; not quantified), posterior dorsal medial amygdala (MePD), and throughout the VTA (Table 1). Although dense TH-ir fibers were found in the AcA, there were no TH-ir cell bodies. The density of TH-ir labeled cells in these brain regions is summarized in Table 3. The only brain region that showed a group difference was the LS ($F_{(3,35)} = 4.50$, $P < 0.05$), in which the baseline control group had a higher density of TH-ir labeled cells than did the other three groups indicated by the SNK post hoc test.

Many TH-ir labeled cells were also found to be co-localized with Fos-ir staining in each of the brain regions examined (Fig. 4). Although similar patterns of group differences were found in TH-ir/Fos-ir labeling in the BNST (Fig. 5B), MPOA (Fig. 5C), and PVN (Fig. 5D) as was observed for the Fos-ir labeling in these brain regions (Fig. 2B–D), significant group differences were only found in the MPOA ($F_{(3,35)} = 3.07$, $P < 0.05$; Fig. 5C) and LS ($F_{(3,35)} = 3.02$, $P < 0.05$; Fig. 5A).

The SNK post hoc test indicated that the MPOA of males exposed to a male intruder had a higher density of TH-ir/Fos-ir cells than did control males, whereas in the LS, baseline control males had a higher density of TH-ir/Fos-ir stained cells than did males exposed to a female intruder. A significant group difference was also found in the AH ($F_{(3,35)} = 7.59$, $P < 0.001$), specifically in TH-ir/Fos-ir double-labeled cells in the areas ventral to the fornix. The SNK post hoc test indicated that the density of cells double labeled with TH-ir/Fos-ir was increased if males were re-exposed to their partner and was significantly increased if males were exposed to an unfamiliar female or male intruder (Fig. 5E). No group difference was found in the MeA and VTA (Fig. 5F,G).

## AVP-ir cells in the AH

AVP-ir cells were found primarily in the nucleus circularis (NC), medial SON (not quantified), and surrounding areas ventral to the fornix in the AH (Fig. 6A). Although no group differences were found in the density of AVP-ir single-labeled cells (Fig. 6B), such differences were found in the density of AVP-ir/Fos-ir double-labeled cells ($F_{(3,35)} = 6.82$ $P < 0.001$; Fig. 6C). The SNK post hoc test indicated that the density of AVP-ir/Fos-ir double-labeled cells was significantly higher in the AH, particularly in the NC and surrounding areas ventral to the fornix, of males that were exposed to an unfamiliar male or female intruder, in comparison with baseline control males or males that were exposed to their familiar partner. The latter two groups did not differ from each other.

## DISCUSSION

Male prairie voles display mating-induced pair bonding. In particular, 24-hour mating induces aggression toward conspecific strangers, and this behavior is long lasting and may play important roles for the maintenance of a monog-

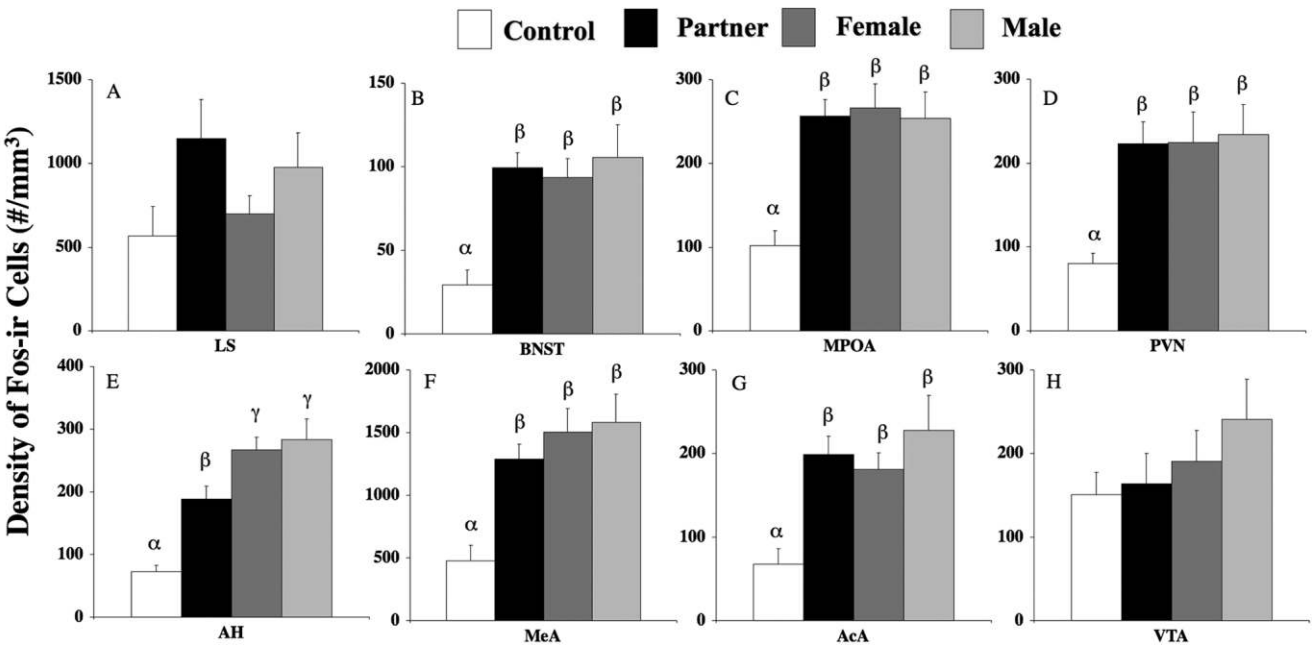


Fig. 2. Pair-bonded male prairie voles exposed to social stimuli had a higher density of Fos-positive cells in the bed nucleus of the stria terminalis (BNST), medial preoptic area (MPOA), paraventricular nucleus (PVN), medial amygdala (MeA), and anterior cortical amygdala (AcA) compared with pair-bonded male prairie voles not exposed to social stimuli (**B–D,F,G**). In the anterior hypothalamus (AH; **E**), pair-bonded males that were exposed to a conspecific male or female stranger had a higher density of Fos-ir labeled cells than control males or males that were exposed to their familiar female partner. No group differences were found in the density of Fos-ir cells in the LS or VTA (**A,H**). Group differences were expressed as $P < 0.05$, and bars with different Greek letters differed significantly from each other. Error bars indicate standard error of the mean.

amous life strategy (Winslow et al., 1993; Aragona et al., 2006). In the present study, we found that male prairie voles that were pair-bonded for 2 weeks displayed aggression toward conspecific male and female strangers, but not toward their familiar mates, and exposure to social stimuli activated several brain areas that have been implicated in processing chemosensory cues and/or in regulating social behaviors. Most interestingly, expression of selective aggression was found to be associated with increased neuronal activation in the AH and in neurons expressing TH or AVP phenotypes. Together, these data not only provide additional evidence supporting the role of the AH in aggression but also suggest that DA and AVP in the AH may serve as neurochemical components in a neural circuit regulating aggression in male prairie voles.

## Selective aggression associated with pair bonding

Previous studies have shown that mating-induced aggression in male prairie voles is selective; it is displayed toward conspecific strangers but not familiar mates (Winslow et al., 1993; Wang et al., 1997; Aragona et al., 2006). Data from the present study provide further evidence to support these findings. Male voles that were pair-bonded for 2 weeks displayed intense aggression selectively toward conspecific male or female strangers but not toward their familiar mate. It should be noted that female prairie voles are induced ovulators (Hairston et al., 2003) and in nature, they display behavioral estrus only by exposure to an unfamiliar male for 24–48 hours (Carter et al., 1987). In previous studies, intact females were used as strangers and therefore were not sexually receptive during the aggression test (Wang et al., 1997; Aragona et al., 2006). In the present study, however, female strangers were estrogen primed to become sexually receptive and as a result less defensive. We found that pair-bonded male voles still aggressively rejected those females, as they did the sexually unreceptive ones (Wang et al., 1997; Aragona et al., 2006), while maintaining a high level of social affiliation with their familiar mates. These data clearly indicate that pair-bonded males can discriminate between their original mate and a potential mate. These data also suggest that sex is unlikely to be the driving force for selective aggression.

Although the underlying mechanism is still unknown, it appears that aggressively rejecting potential mates resembles mate or territory guarding behavior and may play an important role in the maintenance of monogamous pair bonding (Aragona et al., 2006; Young et al., 2005). As a result, the behavioral transition to mating-induced aggression represents a very different manipulation of aggressive behavior than the ones previously reported by using other animal models (Albert et al., 1992; Ferris et al., 1997; Delville et al., 1998; DeLeon et al., 2002b; Ferrari et al., 2003; Miczek et al., 2004; Nelson, 2005; Wommack et al., 2005). Therefore, prairie voles may provide a new and innovative opportunity to study the neurochemical underpinnings of a naturally occurring form of aggression that can be reliably controlled under laboratory conditions.

It should be noted that both offensive and defensive types of aggression are found to be components of selective

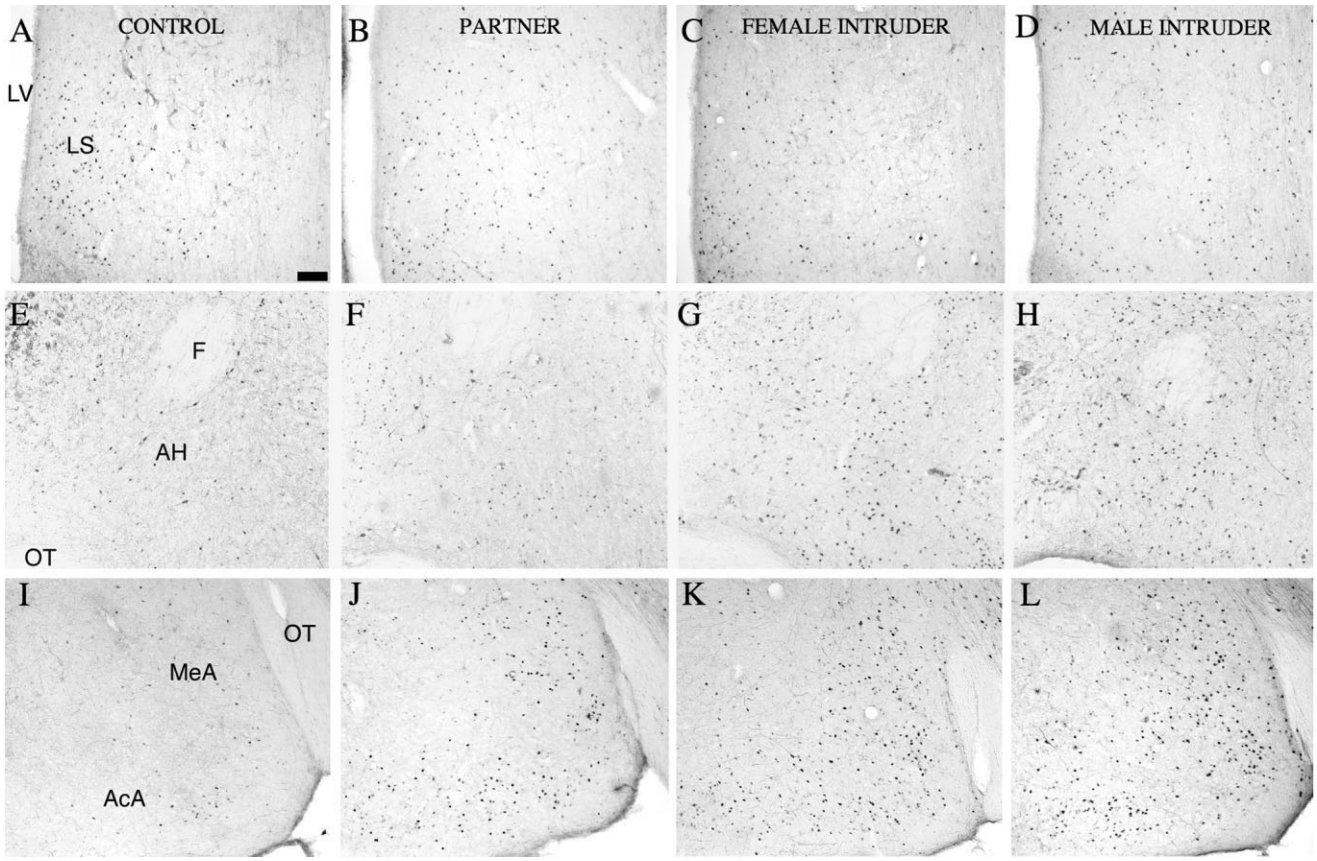


Fig. 3. Photomicrographs of Fos-ir labeled cells in the lateral septum (LS; top row), anterior hypothalamus (AH; middle row), and medial (MeA) and anterior cortical (AcA) nuclei of the amygdala (bottom row) in pair-bonded male prairie voles that did not receive a resident-intruder test (Control: **A,E,I**), exposed to their familiar female partner (Partner: **B,F,J**), an unfamiliar female stranger (Female Intruder: **C,G,K**), or an unfamiliar male stranger (Male Intruder: **D,H,L**). F, fornix; LV, lateral ventricle; OT, optic tract. Scale bar = 100 μm in A (applies to A–L).

TABLE 3. Density of Tyrosine Hydroxylase Immunoreactively Stained Cells (no./mm$^3$)[1]

| Brain area | Control (n = 8) | Partner (n = 9) | Female intruder (n = 10) | Male intruder (n = 12) | F | *P* |
|---|---|---|---|---|---|---|
| LS | 390.60 ± 74.48$^{\beta}$ | 130.81 ± 38.14$^{\alpha}$ | 146.85 ± 36.78$^{\alpha}$ | 198.29 ± 56.95$^{\alpha}$ | 4.50 | <0.05 |
| BNST | 41.52 ± 10.32 | 83.83 ± 12.48 | 78.58 ± 12.99 | 87.04 ± 11.14 | 2.69 | NS |
| MPOA | 96.60 ± 10.88 | 100.33 ± 7.54 | 111.87 ± 10.34 | 120.29 ± 5.87 | 1.51 | NS |
| PVN | 194.64 ± 28.70 | 241.73 ± 29.21 | 238.14 ± 17.12 | 241.03 ± 20.28 | 0.84 | NS |
| AH | 29.88 ± 5.07 | 35.43 ± 5.26 | 47.70 ± 5.29 | 44.84 ± 5.33 | 2.31 | NS |
| MeA | 164.30 ± 19.64 | 172.22 ± 23.91 | 150.55 ± 19.37 | 171.77 ± 19.95 | 0.26 | NS |
| VTA | 446.14 ± 32.10 | 423.04 ± 36.62 | 519.35 ± 40.41 | 493.52 ± 53.00 | 1.13 | NS |

[1]Data are presented as mean ± standard error. Greek letters indicate results of the SNK post hoc test. Groups with different letters differ significantly from each other. NS, not significant. For other abbreviations, see list.

aggression in male prairie voles (Winslow et al., 1993; Wang et al., 1997). In the present study, age- and size-matched intruders were used in order to reduce variability. However, one potential caveat of this design is that the male resident displayed both offensive and defensive types of aggression that could involve different emotional environments and may be regulated by different neural circuitries. Therefore, although our neuroanatomical and neurochemical data (see below) illustrate the potential neuromechanism underlying selective aggression associated with pair bonding, further studies will be needed to better differentiate and characterize each type of aggressive behavior and its underlying neural circuit.

## Neuronal activation following resident-intruder testing

Fos immunoreactivity, an established marker of neuronal activation, has been used previously in vole studies to examine neuronal activity associated with aggression (Wang et al., 1997), maternal (Katz et al., 1999) and paternal behavior (Kirkpatrick et al., 1994), mating (Curtis and Wang, 2003; Lim and Young, 2004), anxiety (Stowe et al., 2005), spatial learning (Kuptsov et al., 2005), chemosensory processing (Tubbiola and Wysocki, 1997; Hairston et al., 2003), social experience (Cushing et al., 2003; Kramer et al., 2006), pharmacological challenges (Ging-

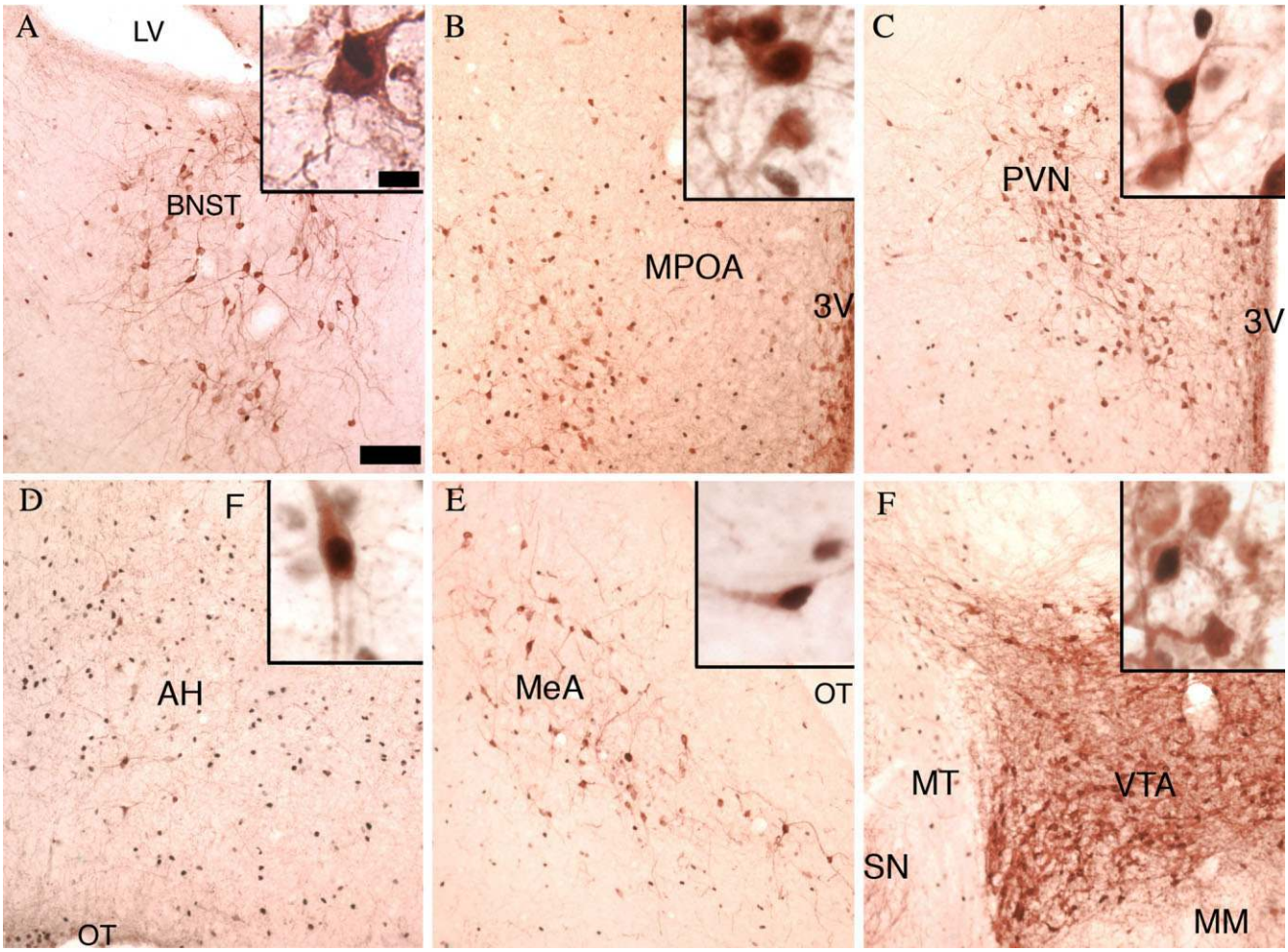


Fig. 4. Photomicrographs of cells labeled for TH-ir (brown cytoplasmic staining), Fos-ir (dark nuclear staining) or both in the bed nucleus of the stria terminalis (BNST; **A**), medial preoptic area (MPOA; **B**), paraventricular nucleus (PVN; **C**), anterior hypothalamus (AH; **D**), medial amygdala (MeA; **E**), and ventral tegmental area (VTA; **F**). F, fornix; LV, lateral ventricle; MM, medial mammillary nucleus; MT, medial terminal optic tract; OT, optic tract; SN, substantia nigra; 3V, third ventricle. The insert within each panel shows cells double labeled for TH-ir and Fos-ir. Scale bar = 100 μm in A (applies to A–F); 10 μm in inset to A (applies to insets to A–F).

rich et al., 1997; Curtis and Wang, 2005b), and photoperiod (Moffatt et al., 1995). However, there is considerable overlap in brain-behavior relationships among these studies, leading to ambiguity as to which brain area(s) play(s) a site-specific role in the regulation of certain types of social behaviors. In the current study, we found that exposure to conspecifics induced increases in Fos-ir expression in several brain areas in pair-bonded male prairie voles, suggesting that chemosensory cues from and/or behavioral interactions with conspecifics resulted in regional increases in neuronal activation in the vole brain. However, different patterns of Fos-ir expression were found among brain areas and after experience with different conspecifics.

In several brain areas including the BNST, MPOA, PVN, and amygdala, males that experienced a resident intruder test showed consistently higher levels of Fos-ir expression in comparison with the baseline control males. However, no group differences were found among males that were exposed to different intruders and/or displayed different patterns of behavior toward intruders. These data suggest that the increased neuronal activation in these brain areas was probably due to olfactory stimulation and/or general arousal associated with exposure to a conspecific, but such a response was nonselective.

Indeed, these findings are supported by existing data. In a previous study (Wang et al., 1997), male prairie voles displayed aggression toward a male intruder only after 24 hours of mating, but not following 24 hours of cohabitation with a female without mating. However, despite their differences in social/sexual experience and in aggressive behaviors, both types of males showed identical levels of Fos-ir expression in some brain areas, such as the BNST. In males that mated for 24 hours, exposure to either a male or a female stranger induced similar increases in the levels of Fos-ir expression in the medial amygdala. Together, these data suggest that a variety of social stimuli and/or behavioral expression may induce similar levels of Fos-ir expression in the above-mentioned brain areas in a broad and nonspecific manner in prairie voles.

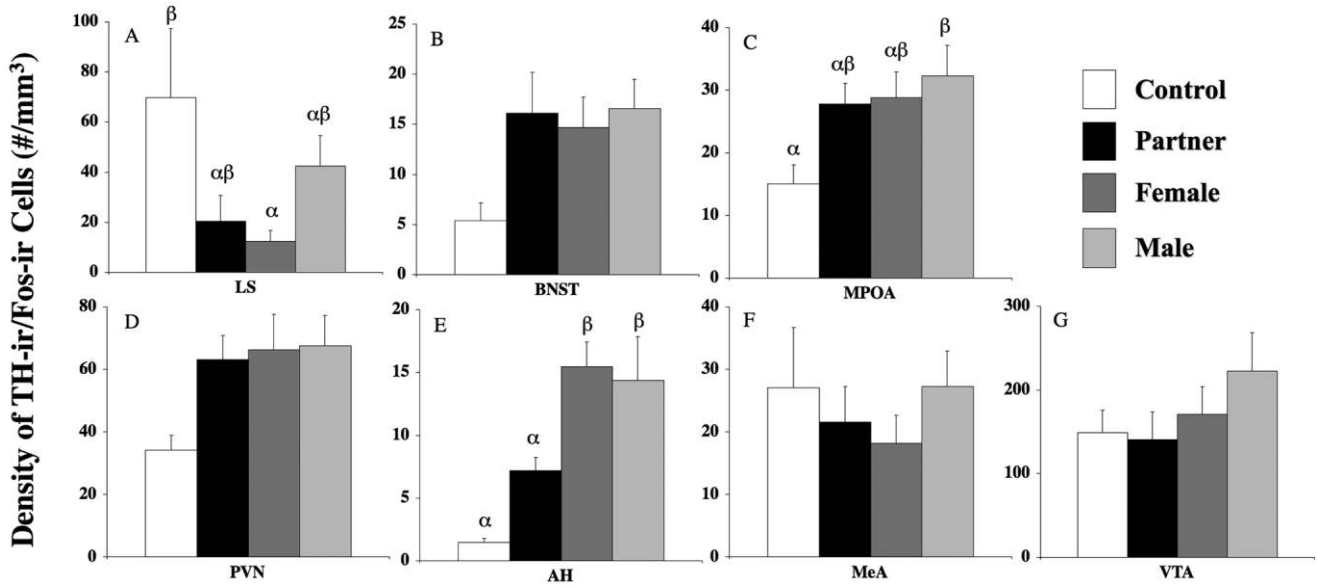


Fig. 5. The density of cells double labeled for TH-ir and Fos-ir in the lateral septum (LS; **A**); bed nucleus of the stria terminalis (BNST; **B**); medial preoptic area (MPOA; **C**); paraventricular nucleus (PVN; **D**); anterior hypothalamus (AH; **E**); medial nucleus of the amygdala (MeA; **F**), and ventral tegmental area (VTA; **G**) in male prairie voles that were pair-bonded for 2 weeks. Males were exposed to their familiar female partner (Partner), an unfamiliar female stranger (Female Intruder), or an unfamiliar male stranger (Male Intruder) during a resident-intruder test. Control males (Control) did not receive resident-intruder testing. Group differences were expressed as $P < 0.05$, and bars with different Greek letters differed significantly from each other. Error bars indicate standard error of the mean.

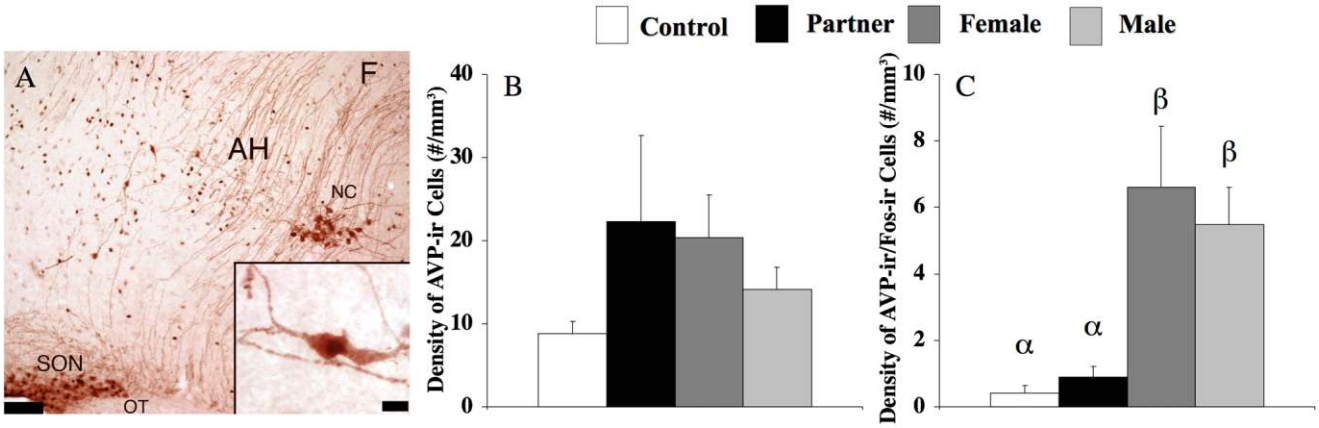


Fig. 6. Cells that were labeled for AVP-ir (brown cytoplasmic staining), or Fos-ir (dark nuclear staining) or both in the anterior hypothalamus (AH) of male prairie voles that were pair-bonded for 2 weeks (**A**). F, fornix; OT, optic tract; NC, nucleus circularis; SON, supraoptic nucleus. The insert illustrates a double-labeled cell for AVP-ir and Fos-ir. These pair-bonded males were exposed to their familiar female partner (Partner), an unfamiliar female stranger (Female Intruder), or an unfamiliar male stranger (Male Intruder) during a resident-intruder test. Control males (Control) did not receive resident-intruder testing. No group differences were found in the density of cells labeled for AVP-ir (**B**), whereas males exposed to a female or a male stranger had a higher density of cells double-labeled for AVP-ir and Fos-ir (**C**). Group differences were expressed as $P < 0.05$, and bars with different Greek letters differed significantly from each other. Error bars indicate standard error of the mean. Scale bar = 100 μm in A; 10 μm in inset to A.

A unique pattern of Fos-ir expression was found in the AH, in which exposure to a familiar partner increased the level of Fos-ir but exposure to a conspecific stranger, either male or female, induced a further and significant increase in Fos-ir. This graded increase in Fos-ir staining may indicate a stimulus-specific response; the AH seems to be more responsive to chemosensory, tactile, and/or visual cues associated with conspecific strangers, but not familiar partners. In addition, males that displayed aggression toward conspecific strangers had an increased density of Fos-ir in the AH in comparison with males that did not display aggression (toward their familiar partner) although all were exposed to conspecific cues. These data indicate that the increased neuronal activation in the AH may be involved in the aggressive behavior displayed by male prairie voles. This notion is corroborated by previous

research documenting the critical role of the AH in regulating aggressive behavior across several mammalian species. For example, the AH has been found to be responsive to specific chemosensory cues from conspecifics that elicit aggressive behavior (Choi et al., 2005; Veening et al., 2005). Electrical stimulation applied directly to a specific area of the hypothalamus (hypothalamic attack area [HAA]) induced attacks toward conspecific animals in rats (Kruk, 1991) and in other animals (Albert and Walsh, 1984; Siegel et al., 1999). In addition, surgical lesioning of the hypothalamus in humans has been shown to reduce aggression significantly (Ramamurthi, 1988; Sano et al., 1966).

Mating and aggressive behaviors were found not to be associated with increased Fos-ir in the LS in male prairie voles (Wang et al., 1997). These findings are confirmed in our present study, as there were no group differences in Fos-ir in the LS between male voles with or without display of aggressive behavior. Although these data indicate the LS as a brain area unlikely to be involved in selective aggression, we cannot exclude the possibility that the LS could be involved in other aspects of pair bonding behavior as it has been implicated in AVP regulation of partner preference formation in male prairie voles (Liu et al., 2001b).

## DA and AVP neurons in the AH are important for selective aggression

In the present study, we mapped the density of TH-ir cells in several forebrain areas and examined colocalization of these TH-ir cells with Fos-ir staining. An interesting pattern emerged in the AH. Males that were exposed to a conspecific male or female stranger had a higher density of cells in the AH that were double-labeled for TH-ir and Fos-ir than baseline control males or males that were exposed to their familiar partner. Similarly, exposure to a conspecific stranger significantly increased the density of cells in the AH that were double-labeled with AVP-ir/Fos-ir, whereas no differences were found between baseline control males and males that were exposed to their familiar partner. These data implicate an involvement of DA and AVP in the AH in processing chemosensory cues related to conspecific strangers and/or in displaying aggressive behavior toward these strangers. In a previous study in hamsters, aggression has been shown to be associated with an increase in AVP-ir/Fos-ir double-labeled cells in the NC, medial SON, and surrounding areas ventral to the fornix in the AH (Delville et al., 2000).

Indeed, AVP in the AH has been implicated in various forms of aggressive behavior in numerous studies across several mammalian species. In hamsters, for example, blockade of AVP receptors in the AH diminished aggression (Ferris and Potegal, 1988; Caldwell and Albers, 2004), whereas intra-AH administration of AVP enhanced aggressive behavior (Ferris et al., 1997; Caldwell and Albers, 2004). More recently, the density of AVP V1a receptors in the AH has been found to increase significantly in Syrian hamsters after social isolation induced aggressive behavior (Albers et al., 2006). Involvement of AH AVP in aggression has also been reported in other rodent species (Zadina et al., 1986). Interestingly, AVP in the AH is found to interact with 5-HT in the regulation of aggression in hamsters (Ferris and Delville, 1994; Ferris et al., 1997; Ferris et al., 1999). In prairie voles, although the AH contains dense labeling of 5-HT terminals (Gobrogge et al., 2005), the interaction between AVP and 5-HT and their roles in aggression are still unknown.

To date, little effort has been made to examine the role of central DA in aggression, and there is only scattered evidence indicating that AH DA may be involved in aggressive behavior. Anatomically, TH-ir fibers and sparse cell bodies exist in the AH of some rodent species (Lansing and Lonstein, 2006). TH-ir fibers have been found to surround and/or to co-localize with AVP-ir cell bodies in the AH (Bridges et al., 1976; Moos and Richard, 1982; Buijs et al., 1984; Lindvall et al., 1984; Chernigovskaia et al., 2001), suggesting a possible functional interaction between AVP and DA in this area. It is worth mentioning that in rats, 6-hydroxydopamine (6-OHDA) lesions in the AH led to a significant reduction of DA levels in the nucleus accumbens (NAcc), caudate putamen, and prefrontal cortex (Winn and Robbins, 1985), whereas intra-AH electrical stimulation differentially exerted cell firing from a specialized group of DAergic neurons in the VTA to the NAcc (Maeda and Mogenson, 1980). These data reveal connections between the AH DA and mesocorticolimbic DA systems and suggest an involvement of AH DA in cognitive and behavioral functions mediated by the mesolimbic DA system. Indeed, previous work examining the effects of cocaine and amphetamine on aggression in rodents (Harrison et al., 2000; Melloni et al., 2001; DeLeon et al., 2002a; Jackson et al., 2005; Knyshevski et al., 2005a,b) indicates a potential influence of central DA systems in the expression of aggression.

## Neural circuit and neurochemical mechanisms involved in selective aggression

DA and AVP involvement in selective aggression has been found in prairie voles. Administration of a DA D1 receptor antagonist into the NAcc diminished selective aggression, revealing effects of NAcc DA on aggression (Aragona et al., 2006). Administration of an AVP V1a receptor antagonist intracerebroventrically blocked mating-induced selective aggression, illustrating central AVP effects on aggression, although the action sites are still unknown (Winslow et al., 1993). Furthermore, the neuroanatomical and neurochemical data from the present study implicate DA and AVP in the AH in selective aggression in male prairie voles. How are these neurochemicals involved in the regulation of selective aggression?

First, selective aggression is a complex social behavior and requires a variety of cognitive functions including, but not limited to, sensory processing, learning and memory, individual recognition, and behavioral output. Therefore, it is not surprising that multiple neurochemical systems are involved in the regulation of selective aggression. In previous studies focused on another pattern of pair bonding behavior, partner preferences, interactions between AVP and OXT (Liu et al., 2001a), DA and OXT (Liu and Wang, 2003; Smeltzer et al., 2006), and DA and glucocorticoids (Curtis and Wang, 2005a), as well as among DA, GABA, and glutamate (Curtis and Wang, 2005b), have been found in prairie voles. Although it was not tested for aggression in prairie voles, viral vector transfer of the prairie vole AVP V1a receptor gene to the ventral pallidum of male meadow voles (*Microtus pennsylvanicus*) increased regional V1a receptor expression and induced partner preference behavior, which, in turn, was blocked by administration of the DA D2 receptor antagonist, im-

plicating interactions between AVP and DA in pair bonding (Lim et al., 2004b).

Other interactions at various levels between DA and AVP may also occur in the regulation of selective aggression, and these interactions may take place site-specifically in the AH. Indeed, it has been shown that DA can regulate AVP release in the hypothalamus (Bridges et al., 1976; Buijs et al., 1984; Lindvall et al., 1984) and reciprocally that AVP administration to striatal rat brain slice preparations significantly increases DA release and cAMP levels (Tyagi et al., 1998). Additionally, both AVP and DA receptors are found throughout various subnuclei of the hypothalamus (Leibowitz et al., 1982; Dubois et al., 1986; Colthorpe and Curlewis, 1996; Albers et al., 2006), although whether or not these receptors are co-localized on the same cell in the AH or anywhere in the central nervous system, to our knowledge, has yet to be described.

Second, complex social behavior can be regulated by multiple brain areas in a neural circuit. For example, male sexual behavior has been shown to be regulated by multiple neurotransmitters in different brain regions in hamsters (Newman, 1999). Unfortunately, due to the lack of evidence in prairie voles, at present we can only speculate. One possibility is that different neurochemicals in multiple brain areas interact, either synergistically or antagonistically, and thus concurrent activations of different neurochemical systems in multiple brain areas of a circuit are essential in the regulation of selective aggression (Liu and Wang, 2003). Another possibility is that different neurochemicals in selected brain areas are activated in a temporal pattern to regulate distinct aspects of selective aggression but that the entire neural circuit is required for complete expression and maintenance of behavior. For example, it is possible that AVP in particular brain areas (still unknown) is involved initially in mating-induced selective aggression (Winslow et al., 1993), whereas DA in the NAcc (and AH) is more important in maintaining the same behavior in males that are pair-bonded for 2 weeks (Aragona et al., 2006). Finally, a previous report in prairie voles showed that using a neutral cage, rather than a resident intruder paradigm, increased aggression in female, but not male, prairie voles (Harper and Batzli, 1997). It would be interesting, in further vole studies, to examine interactions among environmental and neuroendocrine factors and potential sex-specific mechanisms in the regulation of aggression associated with pair bonding.

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