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CART peptide following social novelty in the prairie vole (*Microtus ochrogaster*)

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Abstract

Prairie voles (*Microtus ochrogaster*) are monogamous rodents that display high levels of affiliative behaviors, including pair-bonding, biparental care, and cooperative breeding. Species differences in basal cocaine- and amphetamine-regulated transcript (CART) mRNA and peptide expression have been found between prairie voles and polygamous meadow voles. Therefore, we hypothesized that the CART system may play a role in the regulation of social behavior in this species. Male and female adult prairie voles were placed in a cage either alone, or with a novel social partner of the same or opposite sex. After 45 minutes, subjects were sacrificed and CART peptide expression was examined using immunohistochemistry. We examined fifteen hypothalamic, limbic, and hindbrain regions of interest, focusing on areas that show species-specific patterns of expression. We found that subjects paired with a novel conspecific had lower levels of peptide in the bed nucleus of the stria terminalis (BNST) than isolated animals. This may reflect increased peptide release following increased dopaminergic activity in animals exposed to a novel conspecific. Additionally, CART peptide was higher in the nucleus accumbens (NAc) of subjects paired with an opposite sex partner compared to those paired with a same-sex conspecific, although there was no difference between isolated subjects and either socially housed group. These findings suggest that CART in the NAc is differentially responsive to the sex of adult conspecifics and that the social environment influences CART expression in the prairie vole in a region- and stimulus-specific manner.

Keywords

CART; prairie vole; dopamine; bed nucleus of stria terminalis; nucleus accumbens

1. Introduction

Prairie voles are monogamous rodents that display high levels of affiliative behaviors, including pair-bonding, biparental care, and cooperative breeding (Carter and Getz, 1993). A number of neurotransmitter systems involved in the regulation of these behaviors, including oxytocin (Shapiro and Insel, 1992), arginine vasopressin (Insel et al., 1994), and dopamine (DA; Aragona et al., 2006; Smeltzer et al., 2006), are associated with species-specific

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receptor distribution in prairie voles when compared with closely related polygamous vole species. There are also species differences in basal cocaine- and amphetamine-regulated transcript (CART) mRNA and peptide expression between prairie voles and polygamous meadow voles (Hunter et al., 2005a), suggesting that the CART system may play a role in the regulation of social behavior in this species.

These regional differences in CART expression include increased mRNA and peptide levels in the nucleus accumbens and medial preoptic area, and decreased levels in the bed nucleus of the stria terminalis and cortex of prairie voles, compared to meadow voles (Hunter et al., 2005a). Interestingly, there were few species differences in expression within hypothalamic nuclei. A number of additional areas that show species differences in CART mRNA or peptide expression, as well as those listed above, are implicated in prairie vole social behavior. The species-specific expression in regions known to influence prairie vole social behavior strongly suggests that CART may play a role in the regulation of species-specific prairie vole behavior.

CART peptide is implicated in many behavioral processes including reward processing, anxiety/stress, and feeding behavior via anorectic effects (Hubert et al., 2008; Rogge et al., 2008). CART peptide is involved in reward processing as a proposed homeostatic regulator of DA (Rogge et al., 2008). For example, CART in the NAc blunts the motivational and locomotor response to psychostimulant administration (Jaworski et al., 2003; Jaworski et al., 2008; Kim et al., 2003; Kim et al., 2007). However, most available studies on the relationship between CART and DA involve psychostimulants, and therefore it is unknown whether CART has a homeostatic function in response to natural rewards. A number of species-specific social behaviors in monogamous prairie voles have been closely associated with dopaminergic reward pathways (as reviewed in Young et al., 2008), as well as stress (Bales et al., 2006; DeVries et al., 1995; DeVries et al., 1996). As with the dopaminergic system, there is a positive reciprocal relationship between CART peptides and the hypothalamic-pituitary-adrenal axis (as reviewed in Rogge et al., 2008). CART modulates CRF release (Smith et al., 2004; Vrang et al., 2003) and is responsive to endogenous and exogenous alterations of CRF and glucocorticoids (Hunter et al., 2005b; Stanley et al., 2001; Vicentic et al., 2004; Vicentic et al., 2005).

There are only a few studies implicating CART involvement in social behavior of rodents. Administration of CART peptide into the lateral ventricles or central amygdala of rats reduced social interaction with a novel partner (Chaki et al., 2003; Dandekar et al., 2008). Exposure to the soiled bedding of a female increased c-fos and CART mRNA in the ventral premammillary nucleus of sexually inexperienced male rats (Calvacante et al., 2006). However, these few studies leave much left to explore. Reduced social interaction may be a consequence of increased anxiety rather than a direct effect on social behavior, and Calvacante and colleagues did not actually expose subjects to a conspecific. In this study, we exposed prairie voles to novel conspecifics in order to assess anxiety and social behavior in concert.

Given the species-specific distribution of CART peptide, and the role of CART peptide in both reward and stress, we hypothesized that CART may play a role in social behavior of the prairie vole. Specifically, we exposed male and female voles to either isolation, or a novel social partner of the same or opposite sex. Following testing, we sacrificed the subjects and examined CART peptide expression in a number of brain regions implicated in reward, social behavior, and/or anxiety in rodent studies. Specifically, we hypothesized that CART peptide would be affected by exposure to a novel conspecific in regions that had been previously implicated in prairie vole bonding and affiliation, such as the nucleus accumbens,

lateral septum, bed nucleus of the stria terminalis, medial amygdala, and paraventricular nucleus of the hypothalamus.

2. Results

2.1. Behavior

Among socially paired subjects, there were significant differences in exploratory rearing and non-grooming contact during the testing period. There was a significant main effect of partner status ($F_{2,27}=5.08$, $p=0.03$) and a significant interaction between the subject's own sex and their partner's status ($F_{2,27}=5.98$, $p=0.02$). Males paired with females spent more time engaging in exploratory rearing than all other groups (SS males: $p<0.01$, OS females: $p=0.02$, and SS females: $p=0.03$; Figure 1a).

There were significant main effects of partner status ($F_{2,27}=9.03$, $p<0.01$) and sex ($F_{1,27}=7.95$, $p<0.01$), and a significant interaction ($F_{2,27}=6.66$, $p=0.016$) on physical contact. Females paired with other females had the highest levels of contact, which were significantly higher than all other groups (SS males: $p<0.01$, OS males: $p<0.01$, and OS females: $p<0.01$; Figure 1b).

2.2. CART-ir in regions of interest

CART-ir and sample sizes for each region of interest are presented in Table 1. Of the regions examined, there were significant effects of pairing status on CART expression in the BNST and NAc core, as well as a trend in the NAc shell.

There were significant main effects of each partner status ($F_{2,29}=8.05$, $p=0.001$), and sex ($F_{1,29}=4.05$, $p=0.05$) on CART-ir in the BNST. Males showed higher staining than females (Figure 2). Post-hoc comparisons revealed that isolated animals had significantly higher staining than each of the socially housed groups (SS: $p<0.01$, OS: $p<0.01$; Figure 2). The socially paired groups did not differ from each other ($p=0.69$). Sample sizes are presented in Table 1. Representative micrographs of BNST CART peptide-IR for each group are presented in Figure 4d–f.

There was also a significant main effect of partner status on CART expression in the core of the NAc ($F_{2,42}=4.86$, $p=0.01$). Post-hoc comparisons revealed a significant difference between heterosexually paired and same sex paired individuals ($p<0.01$; Figures 3a), with a trend for a difference between isolated and same sex paired animals ($p=0.06$). Sample sizes are presented in Figure 3a.

Additionally, there was a trend for a main effect of partner status on CART peptide in the shell of the NAc ($F_{2,42}=2.47$, $p=0.09$), with post-hoc tests indicating a significant difference between heterosexually paired and same sex paired individuals ($p=0.03$; Figure 3b). Sample sizes are presented in Figure 3b. Representative micrographs of NAc CART peptide-ir for each group are presented in Figure 4a&b. There were no other significant effects of sex or pairing condition on any other regions examined.

3. Discussion

3.1. Behavioral response to a novel social partner

In the present study, exposure to a novel social partner affected behavior depending on the sex of both the subject and their social partner. Males paired with females spent more time engaging in exploratory rearing around their environment than males paired with other males, or females paired with either sex. These males were not spending less time engaging in other behaviors, however, suggesting that meeting a novel female may increase

environmental exploration or arousal, not avoidance. An additional possible explanation is that these males are displaying increased vigilance, as subjects were within olfactory range of other nearby caged males. Females that were introduced to another female engaged in more non-grooming physical contact than each of the other social groups, suggesting that females may have an increased immediate affiliative response to each other. This is consistent with previous work showing that interactions between novel females are generally affiliative (Bales and Carter, 2003; Bowler et al., 2002).

3.2. Sex differences in CART-ir

CART peptide expression in the regions studied was consistent with previous reports (Hunter et al., 2005a). Of the fourteen regions examined, only one (the BNST) was sexually dimorphic, with males showing higher staining than females (Figure 2). This is consistent with previous research showing that while basal levels of CART peptide are generally not sexually dimorphic (prairie and meadow voles: Hunter et al., 2005a; rats: Balkan et al., 2006; brown antechinus: Ashwell and Mai, 2010), CART response to stimuli may be sexually dimorphic in a region-specific manner (Balkan et al., 2006; Gozen et al., 2007). Although our findings of a sex difference in the BNST may appear to conflict with previous work (Hunter et al., 2005a), it should be noted that this earlier report was qualitative in nature and due to small sample size did not use statistical analyses to verify the presence or absence of sex differences. Of the regions examined, pairing conditions had significant effects on CART peptide expression in both the BNST and NAc, suggesting a role for CART in prairie vole social behavior.

3.3. CART-ir in the BNST

We found that isolated subjects had higher levels of CART peptide in the BNST than socially paired animals, regardless of the sex of the social partner (Figure 2). Prairie voles have lower levels of both CART mRNA and peptide in the BNST compared with meadow voles (Hunter et al., 2005a), supporting a role for BNST CART actions on species-specific behavior. In prairie voles the BNST is responsive to social manipulations (Bosch et al., 2009; Cavanaugh and Lonstein, 2010; Curtis and Wang, 2003; Cushing et al., 2003; Gobrogge et al., 2007; Lei et al., 2010; Lim & Young, 2004; Northcutt and Lonstein, 2009; Wang et al., 1994; Wang et al., 1997). For example, c-fos labeling is increased in the BNST following heterosexual cohabitation both with (Curtis and Wang, 2003; Gobrogge et al., 2007; Lim and Young, 2004; Wang et al., 1997) and without mating (Cushing et al., 2003; Wang et al., 1997). Prolonged heterosexual cohabitation is associated with increases both in arginine vasopressin (Wang et al., 1994) and in CRF mRNA (Bosch et al., 2009) in male prairie voles.

The BNST is primarily involved in stress- and anxiety-related behaviors (Toufexis, 2007; Walker and Davis, 2008). Although the response of CART to stressors has been studied in a number of paradigms (long-term isolation: Dandekar et al., 2009, forced swim test: Gozen et al., 2007; restraint stress: Hunter et al., 2007; Okere et al., 2010) none of these previous studies have examined the BNST. Therefore it is unclear what role, if any, CART in this region plays in response to stress. Our findings raise two non-mutually exclusive explanations: (1) that acute isolation increased CART peptide in the BNST, and (2) that social novelty (regardless of the sex of the subject or partner) decreased CART peptide in this region, presumably due to an increase in peptide release. We will discuss each possibility.

The first possibility is that acute isolation increased CART peptide in the BNST. While acute isolation did not affect the number of c-fos labeled cells in the BNST of prairie voles (3.5 hours: Northcutt and Lonstein, 2009; 24 hours: Stowe et al., 2005), previous studies

using other measures of neuronal activity indicate that the BNST does indeed respond to acute stressors. For example, compared to male prairie voles that were paired with a conspecific, males that were isolated for 3.5 hours in a novel cage displayed a lower number of cells double-labeled for tyrosine hydroxylase (TH) and either c-fos or the transcription factor Egr-1 in the BNST (Northcutt and Lonstein, 2009).

CART is thought to function as a homeostatic regulator of DA (Hubert et al., 2008; Rogge et al., 2008), supported in part by studies finding that drugs of abuse increase CART mRNA (amphetamine: Douglass and Daoud, 1996; cocaine: Fagergren and Hurd, 1999; Hunter et al., 2005b; alcohol: Salinas et al., 2006). Additionally, although intra-NAc administered CART peptide had no effect on locomotor activity, CART co-administration attenuated both cocaine- and DA-induced increases in locomotor activity (Jaworski et al., 2003), suggesting that CART peptide acts downstream from DA receptor activation. Therefore, we would expect to see decreased levels of CART peptide, due to release, in areas of DA receptor activation. However, it is not known whether DA release increases, or DA receptors are activated, in the BNST during pair bonding or other social behaviors in prairie voles.

The second possibility is that social novelty led to increased CART release from the BNST. Dandekar et al. (2008) found that injections of CART directly into the BNST or NAc did not affect behavior in a novel social interaction task. This may be interpreted as contradicting the interpretation that CART peptide in the BNST is responsive to social novelty.

Alternatively, our findings may highlight a species difference in CART response to social stimuli (supported by Hunter et al., 2005a). In prairie voles, exposure to a novel conspecific of either sex increased TH-IR in the posterior BNST, which was not dependent on mating (Cavanaugh and Lonstein, 2010). Given this increase in dopaminergic activity, we would expect to see an increase in CART mRNA and a decrease in CART peptide, reflecting increased CART production and increased CART release, respectively. While we did not measure CART gene expression in this study, this hypothesis is supported by our observation of lower CART-IR in socially-paired subjects (Figure 3a). Male rats exposed to female odors have increased CART mRNA in the ventral premammillary nucleus (Calvacante et al., 2006), a region which has reciprocal connections with the BNST (Contreras et al., 1992; Olmos and Ingram, 1972). It is possible that exposure to odors of a novel social partner in the present study may alter CART activity, with downstream effects on the BNST. Additional studies examining CART mRNA following social novelty may provide valuable insight for understanding potential mechanisms of our results.

3.4. CART-ir in the NAc

CART peptide expression was higher in the NAc of subjects paired with an opposite sex partner compared to those paired with a same-sex conspecific (Figure 3). The differences in CART expression between same-sex and opposite-sex paired animals are intriguing, as these findings suggest that CART in the NAc is differentially responsive to the sex of adult conspecifics. However, it is important to note that there were no significant differences in CART expression in either NAc region between either socially paired group and isolated subjects (although there was a trend in the core, Figure 3a).

When compared with promiscuous meadow voles, prairie voles have higher levels of both CART mRNA and peptide in the core and shell of the NAc (Hunter et al., 2005a), and the NAc has been shown to play a key role in both the formation and maintenance of selective partner preferences in the prairie vole (Aragona et al., 2006; Gingrich et al., 2000; Liu and Wang, 2003). In addition, i.c.v administration of CART increased DA turnover in the NAc of rats (Shieh, 2003; Yang et al., 2004) and overnight isolation following surgery increased striatal concentrations of DOPAC (but not DA or HVA) in female prairie voles (Curtis et al., 2003). Based on these previous data, we would expect to observe decreased levels of CART

peptide in isolated subjects. However, there was no difference in peptide levels between isolates and either socially paired group (although there was a trend for higher CART-ir in the NAc core when compared to subjects paired with a same-sex partner).

Partner preference formation is dependent on NAc DA receptors (Wang et al., 1999). Although mating-induced increases in NAc DA (Curtis et al., 2003) activate D2 receptors, leading to the formation of a pair bond, mating is not required for partner preference formation (Cho et al., 1999; Winslow et al., 1993). Therefore, it is reasonable to hypothesize that heterosexual social contact elicits increases in NAc DA as well, and that increases in CART peptide in this region may reflect this process. Supporting this hypothesis, DOPAC levels were shown to be decreased when females were exposed to a novel male (Curtis et al., 2003). Further studies on CART mRNA following exposure to different social stimuli may provide additional insight into the mechanisms underlying differential CART responses to classes of conspecifics, and may further clarify whether or not these processes are DA-dependent.

There is a positive reciprocal relationship between CART and glucocorticoids (Balkan et al., 2001; Baranowska et al., 2004; Hunter et al., 2005b; Kang et al., 2010; Smith et al., 2004; Stanley et al., 2001; Stanley et al., 2004; Vicentic et al., 2004; Vicentic et al., 2005; Vrang et al., 2003). Both CART mRNA and peptide expression have been shown to be increased in the NAc following corticosterone administration (Hunter et al., 2005b), suggesting that our findings may reflect differences in corticosterone between groups. This is unlikely, as corticosterone levels following social novelty display the opposite pattern than that which would explain our results. Sexually naïve male and female prairie voles showed decreased plasma corticosterone levels when exposed to a novel conspecific of the opposite sex, but not when exposed to a same-sex novel conspecific (DeVries et al., 1997; DeVries et al., 1995). Therefore, changes in NAc DA provide a stronger interpretation of the present study.

3.5. Conclusions

A limitation to our study is that all subjects were exposed to a novel physical and social environment. An additional control group of a familiar same-sex pair being placed in a novel cage would have been informative. An additional consideration is the time of stimulus exposure used in the present study. Although CART peptide expression has been shown to respond to stimuli after as short a time as 16 (Gozen et al., 2007) or 60 minutes (Salinas et al., 2006), many studies employ 2 hour or longer time periods before measuring CART-ir (Hunter et al., 2005b; Kozicz, 2003; Lam et al., 2009). Here we report changes in CART-ir following 45 minutes of social exposure, suggesting that CART peptide responds rapidly to the social environment. It is possible that changes in CART peptide in other regions of interest may emerge in response to novel social stimuli with a longer exposure time. Finally, the sample sizes for some regions are small (see Table 1), and therefore it is possible that we lacked sufficient power to detect group differences for certain brain regions.

We have demonstrated that CART peptide expression in the BNST and NAc is involved in the response to novel social environments. These effects may be mediated by region-specific changes in dopaminergic activity, although the direction of effects observed in the present study seems to conflict with previously published studies on CART and DA. A relevant consideration is that support for the hypothesis that CART functions as a homeostatic modulator of mesolimbic DA is derived from work with large increases in DA induced by psychostimulant administration (as reviewed in Hubert et al., 2008). Therefore it is unclear what the relationship between CART and DA is to more naturalistic rewarding stimuli (such as a social partner), and warrants further investigation. Our findings indicate that the social environment (or lack thereof) influences CART expression in the prairie vole in a region- and stimulus-specific manner.

4. Experimental Procedure

4.1. Subjects

The prairie voles (*Microtus ochrogaster*) used in this study were from an outbred stock originally captured in Illinois and reared at the University of California, Davis. Animals were weaned at 21 days of age and housed in same sex pairs in standard mouse cages (27 cm long × 16 cm wide × 13 cm high) until testing as adults. Colony rooms were maintained under controlled temperature, and light cycles (14L:10D). Food (Purina high-fiber rabbit diet) and water were available *ad libitum*. All procedures were approved and annually reviewed by the Institutional Animal Care and Use Committee of the University of California, Davis. Subjects were adult male and female voles over 60 days old.

4.2. Test Conditions

Each subject was placed in a novel cage under one of three conditions: alone (IS), with a novel same-sex partner (SS), or with a novel opposite-sex partner (OS). Eight animals of each sex were used per group. This protocol has been shown to result in differential c-fos expression based on the sex of the subject and pairing condition (Cushing et al., 2003). Group differences in CART-ir may be detected as soon as 16 or 60 minutes following various conditions (Gozen et al., 2007; Salinas et al., 2006). In the present study, animals were kept in their respective pairing conditions for 45 minutes. Socially housed subjects were videotaped during the entire session. Following the 45-minute testing period, animals were anesthetized under a mixture of ketamine (67 mg/kg) and xylazine (13 mg/kg), sacrificed by cervical dislocation and brains extracted.

Brain tissue was post-fixed by passive perfusion in a spinning mixture of 4% paraformaldehyde and 5% acrolein for 4 hours at room temperature. Following perfusion, brains were stored in 25% sucrose at 4°C until sectioning. Brains were sectioned at 40 µm on a sliding microtome and cryoprotected at -20°C until processing.

4.3. Behavioral observations

All socially-housed pairs were video recorded for behavioral observations. Identifying markers for one female SS pair were damaged during observations and these subjects were therefore not analyzed. Sessions were scored using Behavior Tracker 1.5 (www.behaviortracker.com). For each focal animal the behaviors scored included durations of sniffing the partner, autogrooming, allogrooming, receiving allogrooming, physical contact (excluding grooming bouts) and exploratory cage rearing (Bales and Carter, 2003). The frequency of each aggressive lunges and defensive rearing were also scored.

4.4. Immunohistochemistry

Immunohistochemistry was performed with a modified protocol based on Hunter et al. (2005a). Floating sections were washed in 0.01M KPBS, then pre-treated in 1% sodium borohydride (pH 7.4) for 20 minutes. The tissue was then washed in KPBS, incubated for 15 minutes in 0.014% phenylhydrazine, and washed again. The tissue was incubated in a block solution (1% normal goat serum, 1% bovine serum albumin, and 0.3% Triton-X in KPBS) for 1 hour at room temperature. Following another wash, sections were then incubated overnight at 4°C in a primary antibody solution containing rabbit anti-CART antiserum (1:20,000; Phoenix Pharmaceuticals, Belmont, CA) in block solution.

Twenty-four hours later, sections were washed and then incubated for 1 hour with biotinylated goat anti-rabbit at 1:200 dilution in blocking solution for 90 minutes at 4°C. Tissue sections were then washed and incubated in ABC for 90 minutes at 4°C. Sections were then washed first in KPBS and then in sodium acetate buffer, then staining was

developed using a Ni-DAB reaction for approximately 5 minutes. Sections were then rinsed in sodium acetate buffer and KPBS and mounted, dehydrated, and coverslipped onto glass slides.

4.5. Microscopy and quantification of CART-ir

Photographs of the nucleus accumbens were taken at 10x magnification using a Zeiss AxioCam on a Zeiss Imager A.1. Other regions were photographed using a Micropublisher 3.3 RTV camera on a Leica DM4000B. Photos for each region were taken from a single camera. We examined the following regions of interest for CART peptide expression: the core and shell of the nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), lateral septum, anterior hypothalamus, arcuate nucleus, dorsomedial hypothalamus, lateral hypothalamus, medial preoptic area, paraventricular nucleus of the hypothalamus, ventromedial hypothalamus, central amygdala, medial amygdala, substantia nigra and Edinger-Westphal nucleus. Regions were chosen based on the following criteria: (1) involvement in prairie vole behavior, and/or (2) known region of CART regulation of behavior. For each region, images were converted to 8-bit grayscale and the percentage of immunoreactive area per μm^2 for each region was obtained using the Threshold function of NIH ImageJ software. We aimed to analyze four photos from bilateral sections for each subject and region of interest, although in some cases only two or three sections were analyzed. Within each subject, immunoreactive percentage values were averaged for each region. The sample size for each region varied, based on tissue availability and ranged from 4–8 subjects of each sex per condition.

4.6. Statistical analysis

Each scored behavior was analyzed for each region with a mixed model ANOVA with sex, pairing status, and a sex by pairing interaction. CART-immunoreactive (IR) staining for each region of interest was also analyzed with a mixed model ANOVA with sex, pairing status, and a sex by pairing interaction. Non-significant factors were dropped from the model, if applicable. Post-hoc analyses were made using least square means comparisons between all groups.

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Abbreviations

BNST	bed nucleus of the stria terminalis
CART	cocaine- and amphetamine-regulated transcript
CRF	corticotrophin releasing factor
DA	dopamine
-ir	immunoreactive
NAc	nucleus accumbens

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Highlights

- Isolated subjects have higher levels of BNST CART-ir than those exposed to a novel social partner
- NAc CART-ir is higher in subjects paired with a novel opposite sex partner versus a same sex partner
- Social environment influences CART expression in the prairie vole in a region- and stimulus-specific manner.

Figure 1. Duration of time spent engaging in exploratory rearing (a) and non-grooming physical contact (b) during the 45-minute testing period, by sex and social partner status. Bars indicated significant differences between groups ($p < 0.03$).

Figure 2.

CART peptide immunoreactivity in the BNST for males and females by treatment for three conditions: isolated (IS), novel same-sex partner (SS), and novel opposite-sex partner (OS). Overall, males had significantly higher staining in the BNST than females ($p=0.05$). Isolated subjects had higher CART-ir staining than either socially housed group, with no sex interaction; $*p=0.01$.

Figure 3.

CART peptide immunoreactivity in the NAc core (a) and NAc shell (b), pooled by sex for three conditions: isolated (IS), novel same-sex partner (SS), and novel opposite-sex partner (OS). Sample sizes for each group are presented in parentheses on the horizontal axis. Horizontal bars indicate significant differences between groups; ** $p \leq 0.01$, * $p < 0.05$, # $p < 0.10$.

Figure 4. CART peptide immunoreactivity in the nucleus accumbens (top panel) and BNST (bottom panel) of isolated (IS; a,d), same-sex paired (SS; b,e), and opposite-sex paired (OS; c,f) prairie voles. Subjects that were placed in isolation had higher CART-ir in the BNST than either social paired group. Subjects paired with a novel same sex partner had higher CART-ir in the nucleus accumbens than both heterosexually paired (core and shell) and isolated (core only) subjects.

Percentage of immunoreactive area (mean $\pm$ standard error) for each sex across housing condition

Table 1

Sample size (n) and percentage of immunoreactive area (mean $\pm$ standard error) for each sex across three conditions: isolation, novel same-sex partner, and novel opposite-sex partner. The regions examined are: the core and shell of the nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), lateral septum (LS), anterior hypothalamus (AH), arcuate nucleus (Arc), dorsomedial hypothalamus (DMH), lateral hypothalamus (LH), medial preoptic area (MPOA), paraventricular nucleus of the hypothalamus (PVN), ventromedial hypothalamus (VMH), central amygdala (CeA), medial amygdala (MeA), substantia nigra (SN), and Edinger-Westphal nucleus (EW).

Condition Sex	Isolated				Same-Sex Partner				Opposite-Sex Partner			
	males		females		males		females		males		females	
	n	mean	n	mean	n	mean	n	mean	n	mean	n	mean
Nac core [*]	7	51.9 ± 9.1	6	48.2 ± 7.9	8	64.7 ± 5.8 ^c	8	60.6 ± 3.7 ^c	8	39.2 ± 6.2 ^a	8	48.3 ± 6.1 ^a
Nac shell [*]	7	64.8 ± 9.5	6	67.3 ± 8.9	8	75.6 ± 4.4 ^c	8	74.4 ± 2.5 ^c	8	57.5 ± 7.8 ^a	8	65.5 ± 5.5 ^a
BNST ⁺⁺	5	59.7 ± 4.9 ^{bc}	4	56.7 ± 4.1 ^{bc}	6	49.7 ± 4.2 ^{ac}	6	35 ± 3 ^{ac}	5	42.8 ± 5.6 ^{ab}	7	38.8 ± 5 ^{ab}
LS	6	36 ± 8.7	7	38.8 ± 7.1	8	39.6 ± 7.4	8	36.7 ± 7.9	7	38.5 ± 7.8	8	35.5 ± 6.8
AH	7	7.52 ± 3	6	7.5 ± 1.4	6	8.56 ± 4.7	7	11.7 ± 3.7	7	13.7 ± 3.4	6	7.28 ± 2.1
Arc	4	74.7 ± 17	4	82.2 ± 7	4	79.1 ± 7.5	4	80.1 ± 5.3	4	79.7 ± 7.1	5	72.3 ± 8
DMH	7	31.6 ± 7.7	5	35.5 ± 12	6	26.5 ± 4.9	7	31.2 ± 5.4	7	34.8 ± 6.7	4	28 ± 3.8
LH	6	10.4 ± 3.1	5	4.47 ± 0.7	6	5.86 ± 2.2	6	7.81 ± 2.5	7	5.97 ± 1.4	6	13.9 ± 4
MPOA	6	24.1 ± 5.9	3	9.18 ± 1.7	6	20 ± 8.6	6	24 ± 8.1	6	21.2 ± 7.5	6	18.8 ± 5.4
PVN	7	47.9 ± 8.9	6	35.3 ± 6.8	6	25.7 ± 10	7	44.1 ± 6.6	8	41.8 ± 4.6	6	44.2 ± 8.8
VMH	5	35.6 ± 12	5	32.3 ± 8	7	45.1 ± 14	6	41.4 ± 7.1	6	44.6 ± 7.9	4	24.3 ± 7.8
CeA	7	42.3 ± 11	5	47.9 ± 11	7	59.6 ± 9.3	8	50.8 ± 7.5	7	55.7 ± 7.1	7	50.9 ± 7.1
MeA	7	28.3 ± 7.7	6	28.3 ± 8.6	6	33.3 ± 8	8	38.2 ± 7.8	6	37.6 ± 5.9	5	35.8 ± 11
SN	7	11.3 ± 3.8	7	11 ± 1.9	6	13.5 ± 2.4	6	10.2 ± 1.9	6	17.6 ± 4	4	10.5 ± 2.3
EW	5	19.2 ± 2.1	6	21.3 ± 2	6	19.6 ± 3	5	19.7 ± 1	7	18.7 ± 1.7	5	21.2 ± 1.8

⁺ Main effect of sex (p<0.05).

^{*} Main effect of partner (p<0.05);

^a Significant difference from isolated subjects (p<0.05);

^b Significant difference from the same-sex partner group (p<0.05);

^c Significant difference from the heterosexually paired group (p<0.05).