

Research report

# Species differences in brain distribution of CART mRNA and CART peptide between prairie and meadow voles

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Accepted 13 April 2005

Available online 24 May 2005

## Abstract

Reward mechanisms are involved in pair bond formation in monogamous prairie voles. Given the potential role of CART (cocaine- and amphetamine-regulated transcript) in reward, and its possible role as a third neurohypophysial hormone, we examined the brain distribution of CART mRNA and peptide in monogamous prairie voles compared to congener promiscuous meadow voles. Large species differences in CART mRNA distribution were apparent in the nucleus accumbens, bed nucleus of the stria terminalis, hippocampus, and cortex. CART peptide distribution largely mirrored, but did not exactly match, CART mRNA distribution. Dramatic species differences also existed in CART peptide distribution, including the medial preoptic area, nucleus accumbens, central amygdala, lateral septum, and cortex. In contrast, several brain regions were highly conserved between prairie and meadow voles, including many subnuclei examined within the hypothalamus and olfactory tubercle. Taken together, these data suggest a potential role for CART in the regulation of pair bond formation between monogamous mates and suggest potential brain regions involved in its neural circuitry. Our findings also point to novel avenues of investigation regarding the brain mechanisms for the evolution of diverse social organization.

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*Theme:* Neural basis of behaviour

*Topic:* Comparative neuroanatomy

*Keywords:* Monogamy; Nucleus accumbens; Lateral septum; Central amygdala; Dentate gyrus; Bed nucleus of stria terminalis

## 1. Introduction

Prairie voles (*Microtus ochrogaster*) are wild rodents characterized by monogamous social structure, including pair bond formation between adult mates [15]. In contrast, meadow voles (*Microtus pennsylvanicus*) exhibit the opposite social structure and do not form social attachments between mates [18]. Comparisons of these two vole species

have proven useful in examining the neural basis of pair bond formation [53]. Previous studies have implicated the neurohypophysial peptides vasopressin and oxytocin in the neural regulation of pair bond formation in prairie voles [50,51]. CART (cocaine- and amphetamine-regulated transcript) is a putative third neurohypophysial peptide [41] that is involved in many behaviors, including reward and reinforcement, feeding, stress, and fear-related behavior, and energy homeostasis [12,20,21,24].

Species differences in brain oxytocin and vasopressin systems exist between monogamous and promiscuous vole species [22,23]. These studies allowed the identification of candidate brain regions that differ in oxytocin and vaso-

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pressin systems in prairie and meadow voles, including reward regions such as the nucleus accumbens and ventral pallidum [34]. Subsequent pharmacological manipulations determined the necessity of these reward regions for intact pair bond formation in prairie voles [32,55]. This has led to the hypothesis that pair bond formation is a form of reward learning, in which the rewarding aspects of mating are selectively associated with properties of the partner, much like a “conditioned partner preference” [1,36]. In frog, rat, and non-human primate, CART is found in many of the same reward regions of the brain as vasopressin and oxytocin systems, including the nucleus accumbens, ventral pallidum, and ventral tegmental area [7,31,43,44]. CART injected into the ventral tegmental area induces increased locomotion and conditioned place preference in rats, and thus may be involved in reward and reinforcement and the modulation of mesolimbic dopamine [25,27].

In order to explore the relationship between the neuro-anatomical distribution of CART and natural reward in a wild rodent population, we compared CART distribution between monogamous prairie voles and promiscuous meadow voles. We mapped CART mRNA expression using *in situ* hybridization and CART peptide distribution using immunocytochemistry. Because CART is potentially involved in reward, and reward is involved in pair bond formation, we hypothesized that CART distribution would differ between monogamous and promiscuous voles, with CART enrichment in reward regions in monogamous voles. We also hypothesized that monogamous and promiscuous voles would not differ in brain regions considered important for “classical” CART functions, such as in the hypothalamus where it regulates feeding and energy homeostasis.

## 2. Materials and methods

### 2.1. Subjects

Adult male and female prairie and meadow voles were maintained in a laboratory breeding colony at Emory University. After weaning at 21 days of age, subjects were housed in same sex sibling pairs or trios and water and Purina rabbit chow provided *ad libitum*. All cages were maintained on a 14:10-h light–dark cycle with the temperature at 20 °C. We used a total number of 6 meadow voles and 9 prairie voles for the *in situ* hybridization mapping study, and 4 meadow voles and 3 prairie voles for the immunocytochemistry experiments.

### 2.2. *In situ* hybridization

Animals were deeply anesthetized, rapidly decapitated, and their brains fresh frozen on powdered dry ice and stored at –80 °C. Brain sections were cut at 14 µm on a cryostat and placed on Fisher Biotech ProbeOn Plus slides (Fisher, Pittsburgh, PA).

*In situ* hybridization began with a tailing reaction to radioactively label the CART oligonucleotide probe with <sup>35</sup>S. The probe is directed toward nucleotides 223–270. Processing of the slides followed methods as previously described [7]. Briefly, the slides were incubated in 4% paraformaldehyde (5 min), two washes in 0.1 M PBS (5 min each), dipped in ddH<sub>2</sub>O, 0.1 M TEA, and 0.5% acetic anhydride (10 min), 2 × SCC (3 min), 70% EtOH (3 min), 95% EtOH (3 min), 100% EtOH (3 min), chloroform (5 min), 100% EtOH (3 min), and 95% EtOH (3 min). The slides were then incubated at 40 °C for 2 h with pre-hybridization buffer and coverslipped. Following washes in 2 × SCC and EtOH (70 and 95%), slides were covered with hybridization buffer (~500,000 cpm/slide) and incubated overnight at 40 °C. The next day, the slides were washed 4 × 15 min in 1 × SCC at 55 °C. Slides were brought to room temperature and washed in 0.3 M NH<sub>4</sub>OHac/50% EtOH solution (3 min), 0.3 M NH<sub>4</sub>OHac/85% EtOH solution (3 min), and 100% EtOH (3 min). The slides were then dried and placed on BioMax MR-1 film (Kodak, Rochester, NY) with a <sup>14</sup>C standard slide and exposed in a cassette for about 10–14 days. A number of slides from both species were incubated with a 100-fold excess of unlabeled probe to serve as a control. Optical density was determined using MCID Basic (Imaging Research, St. Catharines, ON, Canada).

### 2.3. Immunocytochemistry

The animals used in the immunocytochemistry (ICC) studies were deeply anesthetized and transcardially perfused with 4% paraformaldehyde (pH 7.4), their brains removed and placed into a 20% sucrose in PBS solution overnight until they sank. Brains were then cut at 60 µm on a freezing microtome and processed as described below.

CART immunoreactivity was visualized with polyclonal antisera (Phoenix Pharmaceuticals, Belmont, CA) raised in rabbit against part of the active fragment, CART 61–102 aa. Sections were processed as previously described [29]. Briefly, the sections were pretreated with sodium borohydride (1% in PBS, 0.01 M, pH 7.4) for 20 min and preincubated with 1% normal goat serum (NGS, Vector Laboratories, Burlingame, CA), 1% bovine serum albumin (BSA; Sigma, St. Louis, MO), and 0.3% Triton X-100 in PBS for 1 h. They were then incubated overnight at 4 °C in the primary antibody solution containing all of the same reagents as above except with the rabbit anti-CART antiserum (1:2000). The sections were washed in PBS 3 × 10 min and incubated at 4 °C for 90 min in the secondary antibody solution with goat anti-rabbit (Vector Laboratories; 1:200 in PBS/1% BSA/1% NGS/0.3% Triton) followed by 3 × 10 min PBS washes and a 90-min incubation at 4°C in the avidin–biotin peroxidase complex (ABC; Vector Laboratories; 1:250 dilution in PBS/1% BSA/0.3% Triton). CART immunoreactivity was revealed with the brown amorphous DAB reaction (0.025%; Sigma, St. Louis,

MO), 0.01 M Imidazole (Fischer Scientific, Pittsburgh, PA), and 0.006% hydrogen peroxide. The reaction was stopped by repeated washes in PBS. The sections were then mounted on gelatin-coated slides, dehydrated, and coverslipped with Permount. Several slides from both species were incubated with an excess of C4 peptide to control for specificity. MCID Basic was used to capture these images on the computer.

### 3. Results

#### 3.1. Species differences in CART mRNA distribution

The distribution of CART mRNA appeared consistent among individual animals within each species. Males did not appear significantly different from females in either species in terms of regional distribution (data not shown). However, there were dramatic species differences in CART mRNA distribution in several brain regions between prairie and meadow voles. These data are summarized in Table 1 as semi-quantitative comparisons of CART mRNA density in several regions throughout the brain. Selected comparisons are shown in Fig. 1.

Prairie and meadow voles differed dramatically in CART expression in reward regions of the brain. Monogamous prairie voles expressed CART mRNA throughout the entire rostral–caudal and dorsal–ventral axis of the nucleus accumbens (NAcc), while the promiscuous meadow voles showed lower expression in the NAcc core (Figs. 1A and B). Similarly, in the ventral pallidum, prairie voles also expressed CART mRNA at a slightly higher level than meadow voles (Figs. 1C and D).

Both vole species also showed dramatic differences in CART expression in other limbic regions of the brain. Monogamous prairie voles showed higher CART expression in the lateral septum (Figs. 1C and D) and medial amygdala (Figs. 1G and H), and claustrum than promiscuous meadow voles. In contrast, meadow voles had much higher CART expression in the taenia tecta (Figs. 1A and B), the lateral division of the bed nucleus of the stria terminalis (Figs. 1C and D), and central nucleus of the amygdala (Figs. 1G and H) than prairie voles.

Several cortical and neocortical regions differentially expressed CART mRNA between vole species. CART mRNA in the somatosensory (Figs. 1A and B) and retrosplenial cortices (Figs. 1G and H) was much more strongly expressed in promiscuous meadow voles than monogamous prairie voles. In contrast, the dentate gyrus

Table 1

CART mRNA levels

Region	Prairie	Meadow
Ventral striatum		
Core	++c	+c
Shell	+++c	++c
Islands of Calleja	+c	+c
Olfactory tubercle	+c	+c
Claustrum	+/-c	0
Ventral pallidum	++c	++c
BnST-LD	+c	++c
Tania tecta	+/-f	++c
Hippocampus		
Dentate gyrus	++c	0
CA3	++c	++c
Cortex		
Piriform cortex	+c	+c
Somatosensory cortex	0	+c
Retrosplenial gyrus	0	+c
Piriform cortex	+	+c
Entorhinal cortex	+/-f	+/-f
Thalamus		
VLN	+/-f	++f
AD	+c	++c
AVN	+f	++f
Habenula	+++c	+++c
LDN	+f	0
LGN	+c	+c
MGN	+f	0
Paraventricular nucleus	+f	0
PPN	+f	+f
VMN	+/-f	+f
Septum		
Laterodorsal	+c	+c
Ventral and intermediate	+/-f	+/-f
VTA	0	+f
Substantia nigra	++c	+c
Hypothalamus		
AHA	++c	+c
AMPO	++c	++c
Arcuate	++c	++c
DMH	+c	++c
LH	+++c	+++c
LM	+c	++c
LPO	+f	+f
MM	++c	++c
MMn	+/-f	+c
MPA	++c	+/-
ME	+++f	+++f
MnPO	+c	+c
PVN	+++c	+++c
PeV	++c	++c
SCH	++c	++c
SON	+++c	++c
SUM	+/-	+
VMH	+f	++f
Zona incerta	+++c	+++c
Amygdala		
Central nucleus	+/-	++c
Basomedial nucleus	+c	+c
Medial nucleus	++c	+c
Posterior cortical nucleus	++c	++c
Basolateral nucleus	+	+
Circumventricular organs		
Subfornical organ	++c	++c
Organum vasculosum	++c	++c

Notes to Table 1:

Semi-quantitative comparison of CART mRNA signal throughout the brain in prairie and meadow voles. Note that there are several regions where prairie voles exceed meadow voles and vice versa. Note also that there are several regions where there are no species differences in CART mRNA density, such as the hypothalamus.

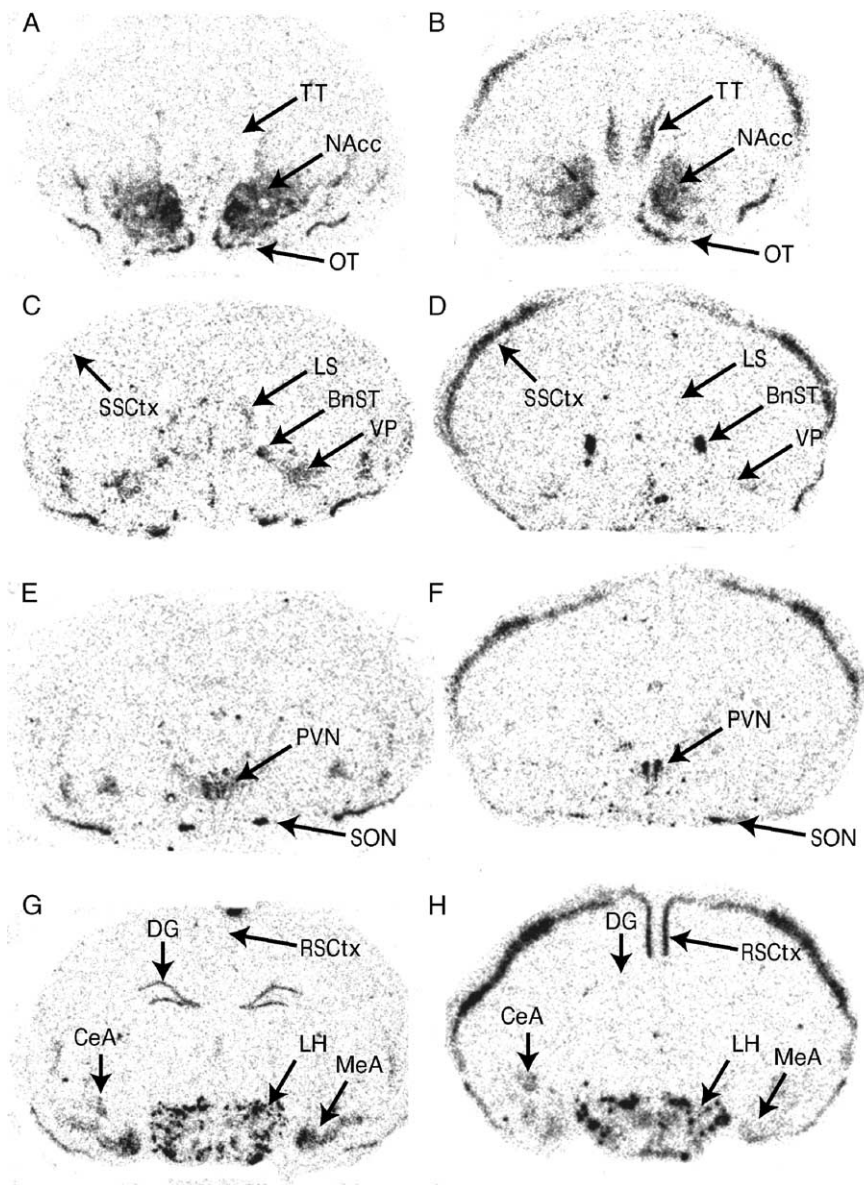


Fig. 1. CART mRNA distribution shown in two vole species using in situ hybridization. Representative brain sections are shown for monogamous prairie voles (A, C, E, G) and promiscuous meadow voles (B, D, F, H). Note the dense CART signal throughout the nucleus accumbens (NAcc) in the prairie vole (A) that is lacking in the NAcc core in the meadow vole (B). In contrast, note the dense CART signal in the somatosensory cortex (SSCtX) in panel B, but not in panel A. In the ventral pallidum (VP), prairie voles (C) have more CART signal than meadow voles (D). Prairie voles (E) and meadow voles (F) both highly express CART in the paraventricular nucleus of the hypothalamus (PVN) and supraoptic nucleus (SON). In the central amygdala (CeA) and retrosplenial cortex (RSCtx), prairie voles (G) express much less CART than meadow voles (H). In contrast, prairie voles (G) more highly express CART than meadow voles (H) in the dentate gyrus of the hippocampus (DG). Scale bar = 1 mm.

subfield of the hippocampus expressed much more CART mRNA in prairie voles than meadow voles (Figs. 1G and H).

Both vole species also showed moderate CART mRNA expression in the piriform cortex and olfactory tubercle (Figs. 1A and B). Both vole species also showed moderate to strong expression in the CA3 region of the hippocampus and the mammillary bodies. Low CART mRNA expression was observed in the habenula and thalamus of either species, with no evident differences. In the hypothalamus, both vole species strongly expressed CART mRNA in a

number of subnuclei. In many nuclei, the expression pattern was very similar, for example, in the paraventricular and supraoptic nuclei (PVN and SON, respectively), which are the two major sources of CART in the brain and periphery, both prairie and meadow voles most highly express CART (Figs. 1E and F). Differences were observed in two regions of the hypothalamus, the medial preoptic area (MPA), and the dorsomedial (DMH) and ventromedial hypothalamic nuclei (VMH). In the MPA, prairie voles show a moderate hybridization signal which is absent in meadow voles (data not shown), whereas in the DMH, meadow voles show a

higher expression level and prairie voles a lower one. In the VMH, this pattern is reversed (Figs. 1G and H).

It should be noted that there are several regions where prairie voles exceed meadow voles in CART expression, and vice versa. Therefore, it is unlikely that there are global species differences in the probe hybridization strength to CART mRNA. In fact, no sex or species differences were observed during the in situ hybridization control experiments, which entailed hybridization of brain sections with the CART sense strand or hybridization of the antisense probe in the presence of a 100-fold excess of unlabeled probe (data not shown). Further, the probe used here is complementary to a highly conserved region of the CART mRNA (100% between rat and mouse).

### 3.2. Species differences in CART peptide distribution

The distribution of CART mRNA was consistent among individual animals within each species. Peptide expression largely mirrored the mRNA distribution with a few exceptions. Semi-quantitative comparisons of regional differences are summarized in Table 2.

Like the mRNA distribution, monogamous prairie voles showed CART-immunoreactivity (CART-ir) in both the shell and core of the nucleus accumbens (NAcc), as well as the islands of Calleja (IC), while promiscuous meadow voles showed similar levels of CART-ir in the NAcc shell and IC but lower ones in the core (Figs. 2A and B). In all three regions, both fibers and light-to-moderate staining cells could be observed (Figs. 3A and B). However, in the ventral pallidum, both prairie and meadow voles showed similarly strong levels of CART-ir (Figs. 2C and D).

The two vole species showed large differences in CART-ir in other limbic regions of the brain. Monogamous prairie voles showed higher CART-ir in the lateral septum (Figs. 2C and D) and medial amygdala (Figs. 2G and H) than promiscuous meadow voles. In contrast, meadow voles had higher CART-ir in the laterodorsal division of bed nucleus of the stria terminalis (Figs. 2C and D) and central nucleus of the amygdala (Figs. 2G and H) than prairie voles. Closer examination (Figs. 4A and B) shows that the central nucleus contains moderate numbers of densely staining cells and fibers in meadow voles but is comparatively void of expression in prairies. In the medial nucleus, this pattern is reversed.

Like CART mRNA distribution, cortical and neocortical regions showed regional differences in CART-ir between vole species. Although much lighter than CART mRNA staining, CART-ir in the somatosensory (Figs. 2C and D)

Table 2  
CART peptide immunoreactivity

Region	Prairie	Meadow
Ventral striatum		
Core	++	0
Shell	+++	++
Islands of Calleja	+	+
Olfactory tubercle	++	++
Claustrum	+	0
Ventral pallidum	+	+/-
BnST-LD	+	+++
Tania tecta	+	++
Hippocampus		
Dentate gyrus	++	0
CA3	++	++
Cortex		
Piriform cortex	+	+
Somatosensory cortex	0	++
Retrosplenial gyrus	0	++
Piriform cortex	++	++
Entorhinal cortex	0	0
Thalamus		
VLN	0	0
AD	+	+
AVN	0	0
Habenula	+	+
LDN	0	0
LGN	++	++
MGN	0	0
Paraventricular nucleus	0	0
PPN	0	0
VMN		
Laterodorsal septum	++	+
Hypothalamus		
AHA	+	+
AMPO	+	+
Arcuate	++	++
DMH	+	++
LH	+++	+++
LM	+	+
LPO	0	0
MM	+++	+++
MMn	+	+
MPA	+	0
ME	0	0
MnPO	+	+
PVN	+++	+++
PeV	++	++
SCH	+	+
SON	+++	+++
SUM	+	+
VMH	++	+
Zona incerta	+++	+++
Amygdala		
Central nucleus	+/-	++
Basomedial nucleus	+/-	+
Medial nucleus	++	+
Posterior cortical nucleus	++	++
Basolateral nucleus	+	+
Circumventricular organs		
Subfornical organ	+	+
Organum vasculosum	+	+

#### Notes to Table 2:

Semi-quantitative comparison of CART immunoreactivity labeling throughout the brain in prairie and meadow voles. CART-ir distribution largely mirrors CART mRNA distribution except in subregions of the thalamus. Note that there are several regions where prairie voles exceed meadow voles and vice versa. c—Immunoreactive cells observed, f—immunoreactive fibers or neuropil only.

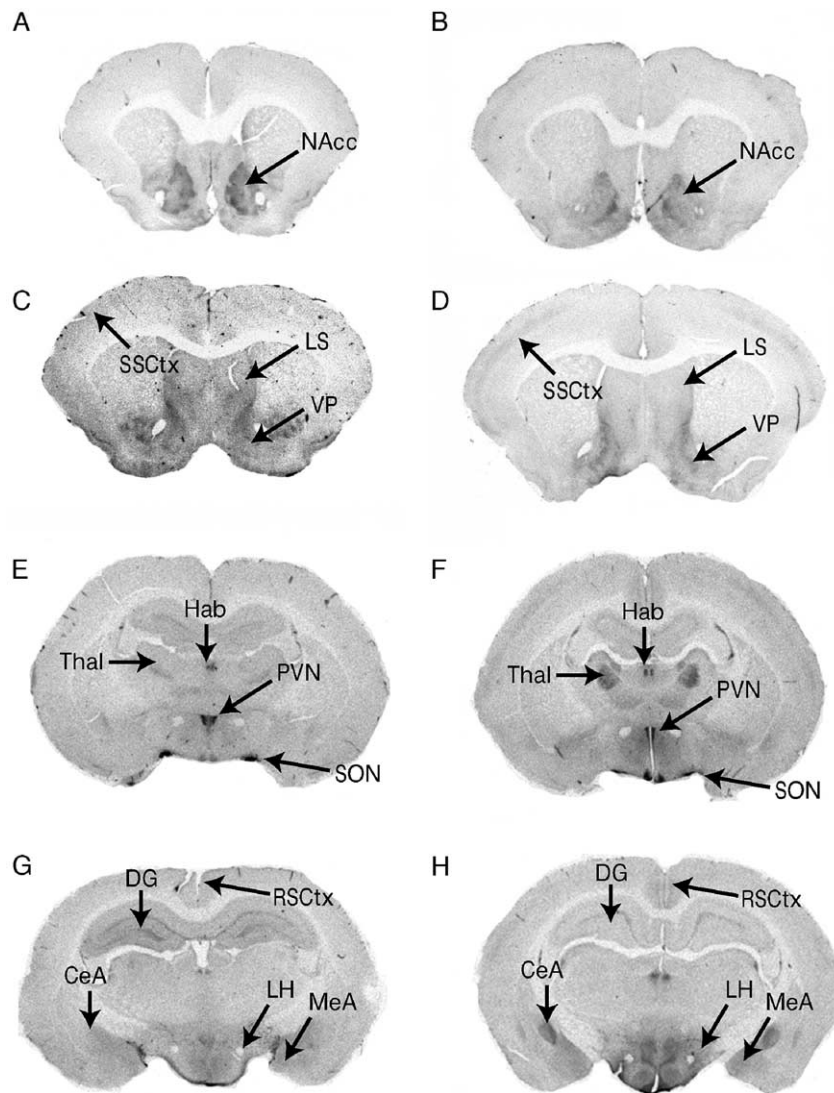


Fig. 2. CART immunoreactivity distribution shown in two vole species using immunocytochemistry. Representative brain sections are shown for monogamous prairie voles (A, C, E, G) and promiscuous meadow voles (B, D, F, H). Note the dense CART labeling throughout the nucleus accumbens (NAcc) in the prairie vole (A) that is lacking in the meadow vole (B). In the lateral septum, prairie voles (C) have more CART-ir than meadow voles (D). Prairie voles (E) and meadow voles (F) both have dense CART-ir in the paraventricular nucleus of the hypothalamus (PVN), supraoptic nucleus (SON), and habenula (Hab); however, only meadow voles show CART-ir in the thalamus (VLThal and VMThal). In the central amygdala (CeA), prairie voles (G) show much less CART-ir than meadow voles (H). In contrast, prairie voles (G) show more CART-ir than meadow voles (H) in the dentate gyrus of the hippocampus (DG). Scale bar = 1 mm.

and retrosplenial cortices (Figs. 2G and H) was also slightly higher in promiscuous meadow voles than monogamous prairie voles. Detailed examination bears this difference out, as seen in Figs. 5A, B, 6A and B. The opposite pattern was true for the dentate gyrus subfield of the hippocampus, which showed higher CART-ir in prairie voles than meadow voles (Figs. 2G and H). Examination at higher magnification shows a population of densely staining cells in the superficial aspect of the granule cell layer of the dentate as well as a population of more moderately staining cells and fibers in the polymorphic layer (Figs. 7A and B).

Both vole species also showed very light CART-ir in the piriform cortex and olfactory tubercle (Figs. 2C and D). Both vole species also showed moderate to strong CART-ir in the CA3 region of the hippocampus and the mammillary

bodies. In the hypothalamus, both vole species had very strong CART-ir in a number of subnuclei. Like CART mRNA, there was strong labeling in the PVN and SON (Figs. 2E and F). Labeling was also observed in DMH, VMH, LH, and arcuate nuclei (Figs. 2G and H) as well as a number of other regions summarized in Table 2. In the MPA, a group of large, densely staining cells was visible in prairie voles but largely absent from meadow voles (Figs. 8A and B). Interestingly, while the pattern of expression in the dorsomedial hypothalamus followed that of mRNA expression (being higher in the meadow vole), the VMH showed a reversed pattern of peptide expression when compared to mRNA levels, as the staining was higher in meadow voles. In both species, the peptide expression in the VMH and DMH is light in cells and appears to be mainly in the

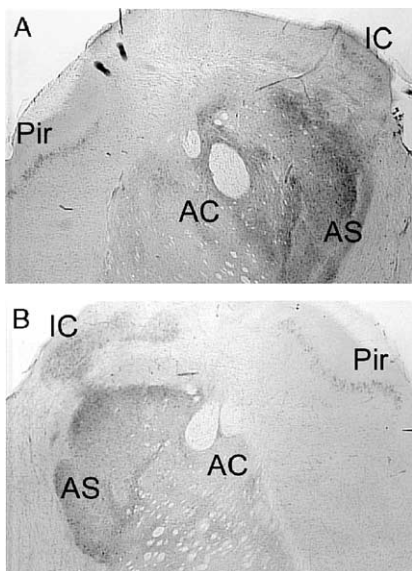


Fig. 3. Photomicrograph showing CART immunoreactivity in the nucleus accumbens of the prairie (A) and meadow (B) vole. AC—NAcc core, AS—NAcc shell, IC—Islands of Calleja.

neuropil (Figs. 9A and B). Moderate differences between the two species were also observed in the supramammillary, lateral mammillary, and medial mammillary nuclei.

Brain regions which showed no or little CART mRNA expression, but did show CART peptide immunoreactivity, included the ventrolateral and ventromedial subregions of the thalamus (Figs. 2E and F), where the staining was principally in fibers and the habenula (Figs. 2G and H) which showed both cell and fiber staining. Meadow voles had much stronger CART-ir in the thalamus, while both

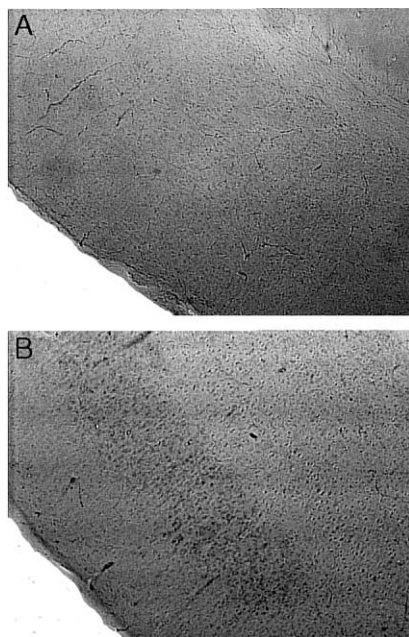


Fig. 5. Photomicrograph showing CART immunoreactivity in the somatosensory cortex of the prairie (A) and meadow (B) vole.

prairie and meadow voles had strong CART-ir in the habenula. Only those nuclei which expressed CART mRNA (the anterodorsal nucleus, the lateral geniculate, and the habenula) showed cellular staining for CART-ir, most of the immunoreactivity observed in the thalamus was found in fibers.

We also performed CART immunocytochemistry in some mid- and hindbrain regions not examined with in situ hybridization (Fig. 10). These data are summarized in

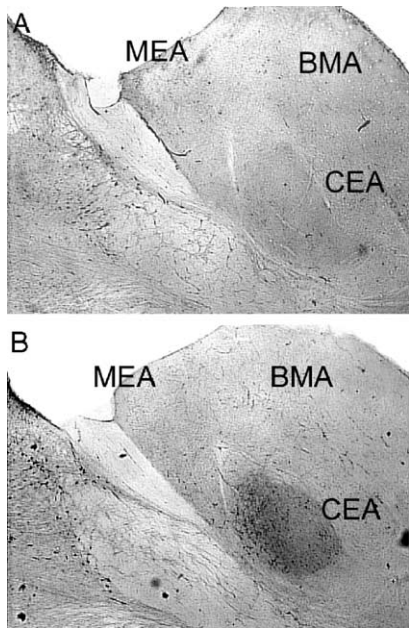


Fig. 4. Photomicrograph showing CART immunoreactivity at the level of the amygdala in the prairie (A) and meadow (B) vole. CEA—central amygdala, MEA—medial amygdala, BMA—basomedial amygdala.

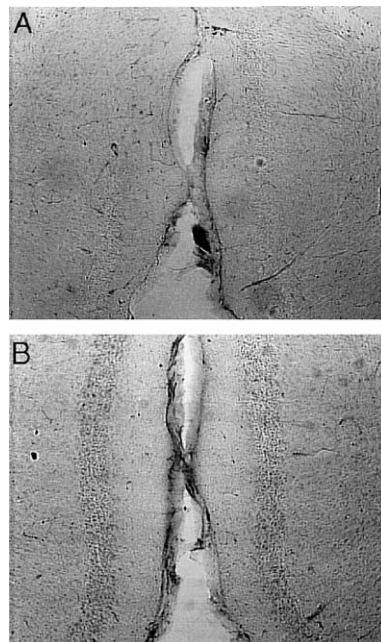


Fig. 6. Photomicrograph showing CART immunoreactivity in the retrosplenial gyrus of the prairie (A) and meadow (B) vole.

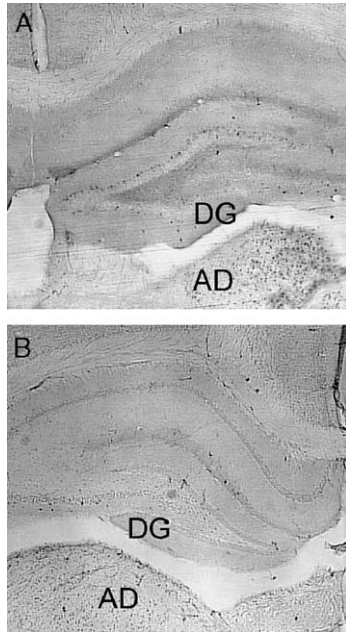


Fig. 7. Photomicrograph of CART immunoreactivity in the dentate gyrus (DG) of the prairie (A) and meadow (B) vole. AD—anterodorsal thalamic nucleus.

**Table 3.** Hindbrain regions examined included the substantia nigra and ventral tegmental area (VTA), where meadow voles showed more light CART-ir signal in the VTA, and prairie voles showed more CART-ir signal in the substantia nigra (Figs. 3A and B). Additionally, strong signal was seen in the superior colliculus, Edinger–Westphal nucleus, and interstitial nucleus of the medial longitudinal fasciculus of both species (Figs. 3A and B). Both vole species also showed moderate peptide levels in the interpeduncular nucleus and periaqueductal gray (Figs.

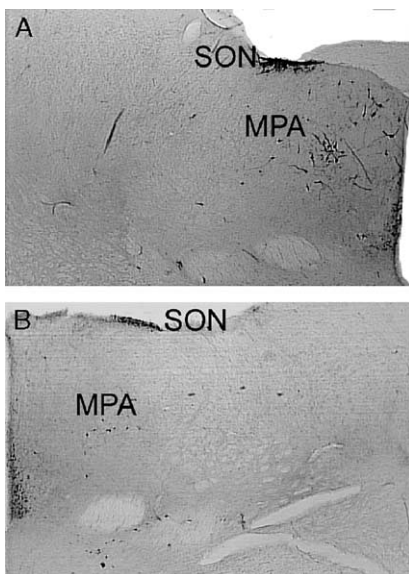


Fig. 8. Photomicrograph showing CART immunoreactivity in the medial preoptic area (MPA) of the prairie (A) and meadow (B) vole. SON—supraoptic nucleus.

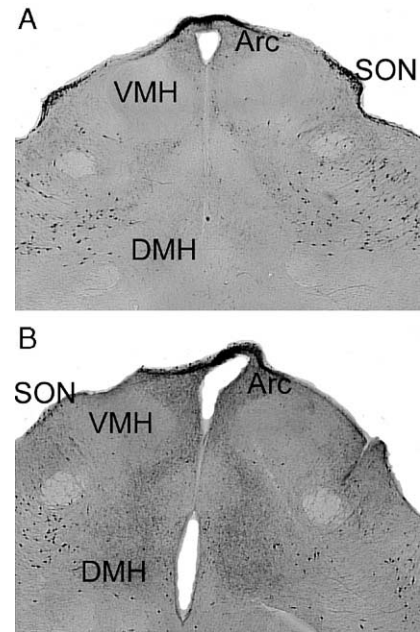


Fig. 9. Photomicrograph showing CART immunoreactivity in the ventromedial hypothalamus (VMH) and adjacent regions. Arc—arcuate nucleus, DMH—dorsomedial hypothalamus, SON—supraoptic nucleus.

3C and 3D). Most caudally, meadow voles showed higher CART-ir in the dorsal tegmental nucleus than prairie voles (Figs. 3E and 3F).

It should be noted that there are several regions where prairie voles exceed meadow voles in CART-ir, and vice versa. Therefore, it is unlikely that global species differences exist in affinity of the CART antibody to the CART peptide. In fact, no sex or species differences were observed in the immunocytochemistry control reactions, which entailed incubation of brain sections with excess CART peptide C4 fragment to quench CART-ir completely (data not shown). Observed background was quite low. Further, the C4 antibody, which was raised against the 79–102 region of the short form of the rat prepropeptide [see Ref. [29] for explanation of antibody nomenclature], has been used successfully in several primate and rodent species [5,14,28,42].

#### 4. Discussion

In these experiments, we mapped CART mRNA and CART peptide distribution in monogamous prairie voles and promiscuous meadow voles and found extensive regional differences throughout the brain. Monogamous prairie voles had more CART labeling in reward regions of the brain, including the NAcc core and ventral pallidum. Prairie voles also had more CART in the MPA, lateral septum, dentate gyrus, and medial amygdala than meadow voles. Promiscuous meadow voles, on the other hand, had more CART in the cortex, bed nucleus of the stria terminalis, and the central amygdala. These species differences in CART distribution

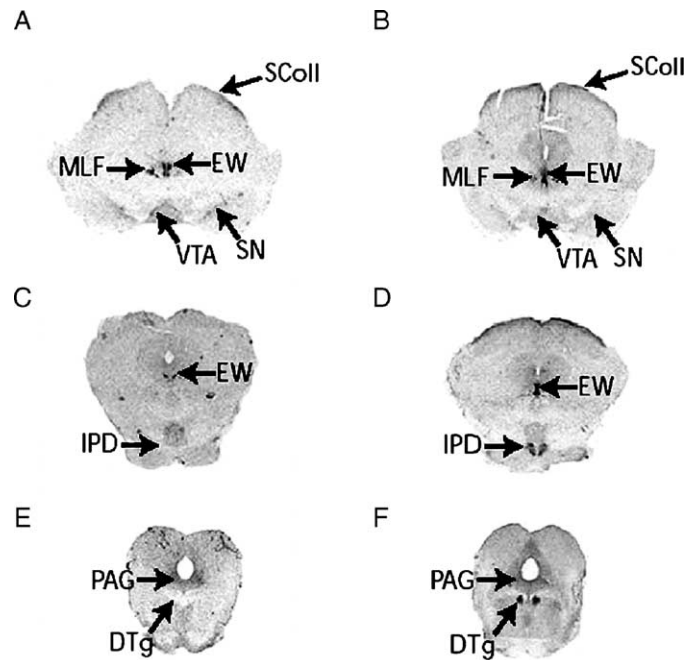


Fig. 10. CART immunoreactivity distribution in the hindbrain shown in two vole species. Representative hindbrain sections are shown for monogamous prairie voles (A, C, E) and promiscuous meadow voles (B, D, F). Note CART-ir in the superior colliculus (SColl), Edinger–Westphal nucleus (EW), medial longitudinal fasciculus (MLF), ventral tegmental area (VTA), and substantia nigra (SN) in the prairie vole (A) compared to the meadow vole (B). Prairie voles (C) had less CART-ir labeling than meadow voles (D) in the interpeduncular nucleus (IPD). Prairie voles (E) also had less CART-ir than meadow voles (F) in the dorsal tegmental nucleus (DTg). Scale bar = 1 mm.

suggest a potential role for CART in the regulation of social behavior and monogamous social organization.

CART is expressed in several regions already implicated in pair bond formation in prairie voles. The nucleus accumbens (NAcc), MPA, lateral septum, and ventral pallidum are all necessary for intact partner preference in prairie voles, and prairie voles express more CART than meadow voles in all these regions [1,16,32,37,55]. The NAcc and ventral pallidum, in particular, are key relay nuclei in the mesolimbic dopamine reward pathway. Reward in these regions has been implicated in pair bond formation, analogous to conditioned place preference [1,36]. Given the role of CART in facilitating conditioned place preference [27], it is possible that CART interacts with dopaminergic

systems in the NAcc to regulate pair bond formation, though this awaits empirical confirmation. The differences observed in the medial preoptic area are also of interest as this area is implicated in pair bonding and maternal behavior [33,38,39].

Monogamous prairie voles also more highly express CART in the dentate gyrus subfield of the hippocampus and the medial amygdala. The dentate gyrus and medial amygdala are regions involved in spatial and social memory, which are also important components of pair bond formation in prairie voles [40,49], and these regions also more highly express CART in prairie voles. Species differences in these brain regions, coupled with reward regions, identify potential neural circuits for CART-dependent pair bond formation.

Vole species differences in CART also appear in regions implicated in anxiety and fear learning, such as the bed nucleus of the stria terminalis (BnST) and the central amygdala (CeA). The anxiety and learning functions of the BnST and CeA have been linked to glucocorticoids and the HPA system. Past studies have shown that glucocorticoids and corticotropin-releasing factor (CRF) both regulate CART expression in the rat brain [2,3,45–47]. Interestingly, both glucocorticoids and CRF also regulate pair bond formation in monogamous prairie voles [9–11]. Thus, it is possible that HPA axis effects on pair bonding are mediated in part through interactions with the CART system.

Several brain regions were highly conserved in CART distribution between vole species. Various subnuclei of the

Table 3  
Relative comparison of CART peptide hindbrain distribution in prairie and meadow voles

Subgroup	Region	Abbreviation	Prairie	Meadow
Hindbrain	Ventral tegmental nucleus	VTA	0	+
	Substantia nigra	SN	++	+
	Superior colliculus	SColl	++	++
	Edinger–Westphal nucleus	EW	+++	+++
	Medial longitudinal fasciculus	MLF	+++	+++
	Interpeduncular nucleus	IPD	++	++
	Periaqueductal gray	PAG	++	++
	Dorsal tegmental nucleus	DTg	0	+++

Semi-quantitative comparison of CART immunoreactivity labeling in the hindbrain in prairie and meadow voles.

hypothalamus expressed CART to the same degree in prairie and meadow voles, including the PVN, SON, DMH, VMH, LH, and arcuate nucleus. All these regions express CART in frog, rodent, and non-human primate as well [8,29,31]. The fact that hypothalamic CART distribution is so highly conserved suggests that CART in these regions regulate conserved physiological functions, perhaps some of which are “core” functions of CART in mammals. Hypothalamic CART is involved in feeding and energy metabolism [21]. CART in the PVN and SON projects to the pituitary where it is released into the periphery in a diurnal rhythm, possibly as a putative third neurohypophyseal peptide [41,46]. The distribution of CART in these nuclei appears highly conserved across mammalian (and even non-mammalian) species and therefore is probably not related to pair bond formation, per se.

Past experiments using voles have demonstrated a role for neuropeptide receptor distribution, in particular vasopressin V1a receptors (V1aR), in regulating social organization [36]. In all the neuropeptides examined (V1aR, oxytocin receptor, CRF receptors 1 and 2), the neuropeptides themselves tend to be highly conserved between species, whereas receptor distribution is highly plastic and variable between species [22,23,35,48]. Therefore, it is very interesting that CART, a large neuropeptide with high sequence conservation between rat and human [17], can have such dramatically variable mRNA and peptide distribution between two closely related vole species. Unfortunately, a CART receptor has not yet been identified, so mapping the CART receptor between vole species cannot be performed as of yet.

What could cause these large species differences in regional CART distribution in the brain? Past work with vasopressin in voles has demonstrated that species differences in V1aR distribution in the brain are likely due to species differences in the promoter region of the prairie vole *V1aR* gene [19,54]. It is possible that the CART story in voles is a similar phenomenon. The CART promoter sequence has been characterized for rat, mouse, and human [4]. The 5' flanking region of the CART gene in human is highly polymorphic and nominally linked to obesity [52] and alcoholism [26] in certain populations, suggesting a possible impact on the function of the human reward system. It is possible that similar polymorphisms exist in the 5' regulatory region of the CART gene between prairie and meadow voles that may partially explain regional differences in the brain.

Comparison of the expression pattern of CART in voles with those previously observed in rats [6,13,28,30] shows a pattern of expression which is in most regards similar. Some differences are worthy of note though. Prairie voles show no CART expression in the somatosensory or retrosplenial cortex, whereas both meadow voles and rats do. On the obverse, neither meadow voles nor rats show much CART expression in the MPA, whereas prairie voles do, which may relate to the presence

of pair bonding and bi-parental behavior in the prairie vole. Meadow voles lack CART expression in the dentate gyrus and have lower levels in the accumbens, while CART is present at higher levels in these regions of prairie vole and rat brains, which suggests the difference may have implications for social memory and reward. The two vole species differ from the rat in that they express CART mRNA and peptide in the cells of the supra-chiasmatic nucleus, while rats only show moderate levels of immunoreactivity in fibers. In general, the expression differences between the three species seem consistent with the idea that CART may have a role in the social behavior of different mammalian species.

Our findings indicate that CART distribution in the brain can vary dramatically even between two closely related species with differing social organization. Potential physiological functions of CART beyond reward, feeding, stress, and energy homeostasis should be extended to consider the regulation of social behavior. However, the true implications for CART in the neural circuitry underlying monogamous social behavior remain to be determined with pharmacological and genetic studies.

## Acknowledgments

This research was supported by DA10732, DA00418, RR00615, and DA015277 to RGH, KBP, and MJK, NIH MH6505 and NSF STC IBN-9876754 to MML and LJY, and the Yerkes Center Grant RR00165.

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