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Species and Sex Differences in Brain Distribution of Corticotropin-Releasing Factor Receptor Subtypes 1 and 2 in Monogamous and Promiscuous Vole Species

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Abstract

Corticotropin-releasing factor (CRF) receptor subtypes 1 and 2 have been implicated in rodent models of anxiety, but much less is known about the CRF system and social behavior. Both corticosterone and central CRF receptors modulate pair bonding in the monogamous prairie vole. Using receptor autoradiography, we mapped CRFR₁ and CRFR₂ in the brains of two monogamous vole species, the prairie vole and pine vole, and two promiscuous vole species, the meadow vole and montane vole. We found markedly different patterns of brain CRFR₁ and CRFR₂ binding among the four species, including species differences in the olfactory bulb, nucleus accumbens, lateral septum, hippocampus, laterodorsal thalamus, cingulate cortex, superior colliculus, and dorsal raphe. Interestingly, we also observed striking sex differences in voles: CRFR₂ binding was higher in the encapsulated bed nucleus of the stria terminalis in males than females for all four vole species. These results suggest possible sites of action for CRF-induced facilitation of pair bond formation in prairie voles, as well as potential sex differences in the CRF modulation of pair bonding. Further examination of CRF receptors in vole species may reveal a novel role for CRF in social behavior. Ultimately, our results identify several brain regions with conserved CRF receptor patterns across rodent and primate species, in contrast to several brain regions with phylogenetically plastic CRF receptor patterns, and have interesting implications for the evolution of CRF receptor patterns and behavior.

Indexing terms

corticotropin-releasing factor; corticotrophin-releasing hormone; nucleus accumbens; lateral septum; hippocampus; bed nucleus of the stria terminalis

Microtine rodents offer a well-established animal model for the comparative study of social behaviors (Insel and Young, 2001; Young and Wang, 2004). Prairie voles (*Microtus ochrogaster*) and pine voles (*Microtus pinetorum*), which exhibit monogamous social structure in nature, show species-typical social behaviors such as pair bonding, affiliation, and biparental care (Getz et al., 1981; Gruder-Adams and Getz, 1985). In contrast, congener meadow voles (*Microtus pennsylvanicus*) and montane voles (*Microtus montanus*) show a strikingly opposite social structure, demonstrating behaviors such as promiscuity, social isolation, and minimal paternal care of offspring (Salo et al., 1993; Shapiro and Dewsbury, 1990). Genetic differences that have evolved between prairie, pine, meadow, and montane voles are thought to contribute to species differences in social organization and brain receptors; these species differences in

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social behavior have been linked to the brain distribution of the neuropeptide receptors for oxytocin and vasopressin (Insel and Shapiro, 1992; Insel et al., 1994; Lim et al., 2004b; Young et al., 1999). Much of the research in the last decade has focused very closely on these neurohypophysial peptides and pair bonding in monogamous prairie voles. However, more recent research has uncovered another candidate neuropeptide, the corticotropin-releasing factor (CRF) system, which also appears to regulate pair bonding in monogamous prairie voles (DeVries et al., 2002).

There is extensive literature linking CRF to stress, anxiety, and the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. However, there are relatively few studies implicating the CRF system in social behavior. One study using prairie voles found that exogenous corticosterone administered to male prairie voles facilitated pair bond formation (DeVries et al., 1996). This effect was mimicked by swim stress, which had been previously shown to activate the HPA axis in voles, prior to pairing (DeVries et al., 1995). Adrenalectomy blocked pair bond formation, and this effect was rescued by corticosterone replacement (DeVries et al., 1996). Interestingly, the opposite phenomenon was observed in female prairie voles, where exogenous corticosterone actually *inhibited* partner preference formation (DeVries et al., 1996). Since CRF released from the paraventricular nucleus of the hypothalamus causes the release of ACTH from the anterior pituitary, and in turn corticosterone from the adrenal cortex, these data suggest that CRF release may play a role in stress-induced activation of the HPA axis during pair bond formation. However, a later study by the same group found that CRF administered intracerebroventricularly (i.c.v.), at doses too low to facilitate anxiety, were sufficient to facilitate partner preference in male prairie voles (DeVries et al., 2002). Central administration of alpha-helical CRF, an antagonist that binds to both CRFR₁ and CRFR₂, blocked partner preference in males (DeVries et al., 2002). These data suggest that CRF may play a role in pair bond formation via anxiety-independent mechanisms and through the involvement of centrally acting brain receptors.

Since the CRF system has been shown to be critical for the regulation of pair bonding, one might predict that neural circuits for this peptide system would differ between monogamous and promiscuous species. In addition, because corticosterone exerts opposite effects on pair bond formation in male and female prairie voles, one might predict that sex differences could potentially exist at the level of central CRF receptors in the brain. Given that corticosterone can exert canonical feedback at both hypothalamic as well as suprahypothalamic brain regions, it is possible that corticosterone could affect CRF release centrally within the brain and, thus, depending on gender-specific CRF receptor expression, activate different neural circuits in male versus female pair bond formation (McEwen et al., 1968; Sapolsky et al., 1990). To test these hypotheses, we first mapped the distributions of CRFR₁ and CRFR₂ in male and female prairie and meadow voles using receptor autoradiography. As an additional axis of comparison, we mapped CRFR₁ and CRFR₂ brain distribution in a different species pair, the monogamous pine vole and the promiscuous montane vole. We show evidence for both species and sex differences in several brain regions that could be related to the differences in their social structure across the four species. Based on a semiquantitative comparison of CRF receptor distributions found in the four vole species to rat, mouse, and rhesus macaque distributions found in previous studies (Aguilera et al., 1987; De Souza et al., 1985; Potter et al., 1994; Primus et al., 1997; Rominger et al., 1998; Sanchez et al., 1999; Steckler and Holsboer, 1999; Van Pett et al., 2000), we discuss putative brain CRF systems that appear to be 1) evolutionarily conserved across species, 2) plastic between species, and 3) potentially associated with monogamous social organization.

MATERIALS AND METHODS

Subjects

Animals were adult male and female prairie voles (70–100 days of age) from our laboratory breeding colony that were originally derived from field-captured voles in Illinois, USA. Adult male and female meadow voles of the same age were derived from stock obtained from the laboratory breeding colony of Dr. Zuoxin Wang at Florida State University. Adult male and female pine voles were obtained from the laboratory breeding colony of Dr. John Vandenberg at North Carolina State University. Montane vole subjects were adult males and females from our laboratory breeding colony originally derived from stock obtained from the National Institutes of Health. 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 light:dark cycle with the temperature at 20°C. Thirty-two animals were used in the generation of data for these experiments ($n = 8$ per species of vole, and four males and four females per species). All experiments were approved by the institutional guidelines set by the animal care and use committee of Emory University and conformed to the guidelines set by the NIH.

CRF receptor autoradiography

Due to the lack of high-affinity iodinated ligands selective for the CRFR₂ subtype, we performed receptor autoradiography with [¹²⁵I-Tyr⁰]-sauvagine, a ligand with high affinity for both CRFR₁ ($K_d = 0.2\text{--}0.4$ nM) and CRFR₂ ($K_d = 0.1\text{--}0.3$ nM) (Grigoriadis et al., 1996; Primus et al., 1997). An incubation period of 2 hours was shown to be optimal for rhesus macaque brain tissue (Sanchez et al., 1999). To identify CRFR₂ receptor binding sites, we combined [¹²⁵I-Tyr⁰]-sauvagine with an excess of unlabeled CP-154,526, a selective CRFR₁ small molecule antagonist (Schulz et al., 1996). To identify CRFR₁ receptor binding sites, we used subtraction of optical density readings of total CRF receptor binding minus specific CRFR₂ receptor binding. The subtraction technique is further described in the Data Analysis section (below).

Animals were deeply anesthetized with isoflurane (10 drops isoflurane placed onto a cotton ball within a chamber measuring 27 cubic inches), rapidly decapitated, their brains removed, and snap-frozen in dry ice. Brains were sliced on a cryostat at 20 μ m, sections thaw-mounted onto Superfrost Plus slides (Fisher, Pittsburgh, PA), and stored at -80°C until use. A 1:7 series of sections were collected from the olfactory bulbs through the hindbrain. Three adjacent sets of sections were processed for CRF total binding (Fig. 1A,B), CRFR₂ autoradiography (Fig. 1C,D), and CRF receptor nonspecific binding (Fig. 1E,F), similar to previously described protocols (Lim et al., 2004a; Sanchez et al., 1999; Skelton et al., 2000).

Slides were thawed at room temperature until completely dried and lightly fixed for 2 minutes in a 0.1% paraformaldehyde-PBS solution (pH 7.4). Slides were then rinsed twice in 50 mM Tris base (pH 7.4) solution for 10 minutes each, then incubated in tracer for 2 hours. The tracer buffer consisted of a 50 mM Tris base, 10 mM MgCl₂, 0.1% bovine serum albumin, 0.05% bacitracin, plus 0.2 nM [¹²⁵I-Tyr⁰]-sauvagine (PerkinElmer/NEN, Boston, MA). This tracer binds both CRFR₁ and CRFR₂. Slides were then rinsed with 50 mM Tris base plus 10 mM MgCl₂ (pH 7.4) for 4×5 minutes, plus 30 minutes with stirring with a magnetic bar on a stir plate. Slides were then dipped in deionized H₂O, blown dry with cool air, and apposed to Kodak MR film for 85 hours with [¹²⁵I] microscale standards (PerkinElmer/NEN). Representative brain sections are shown in Figure 1A,B.

CRFR₂ autoradiography

An adjacent set of slides were processed for CRFR₂ autoradiography. CRFR₂ binding was measured by incubating [¹²⁵I-Tyr⁰]-sauvagine, which binds to both CRFR₁ and CRFR₂, with unlabeled CP-154,526-1 (butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]-pyrimidin-4-yl]-ethylamine), a selective CRFR₁ antagonist which was synthesized at Emory University and kindly provided by Michael J. Owens, Ph.D. This CP-154,526 compound has a high affinity for CRFR₁ sites, with K_i of 2.7 nM, but has low affinity for CRFR₂ sites (Schulz et al., 1996). The concentration of CP-154,526 used in this study (1 μM) would compete with [¹²⁵I-Tyr⁰]-sauvagine for CRFR₁ binding sites, but not CRFR₂ binding sites, because the K_i for inhibition of binding by [¹²⁵I-Tyr⁰]-sauvagine to CRFR₂ is greater than 10 μM (Schulz et al., 1996).

Slides were thawed at room temperature until completely dried and lightly fixed for 2 minutes in a 0.1% paraformaldehyde-PBS solution (pH 7.4). Slides were then rinsed twice in 50 mM Tris base (pH 7.4) solution for 10 minutes each, then incubated in tracer for 2 hours. The tracer buffer consisted of a 50 mM Tris base, 10 mM MgCl, 0.1% bovine serum albumin, 0.05% bacitracin, plus 0.2 nM [¹²⁵I-Tyr⁰]-sauvagine (PerkinElmer/NEN) and 1 μM CP-154,526. Slides were then rinsed with 50 mM Tris base plus 10 mM MgCl (pH 7.4) for 4 × 5 minutes, plus 30 minutes with stirring with a magnetic bar on a stir plate. Slides were then dipped in deionized H₂O, blown dry with cool air, and apposed to Kodak MR film for 85 hours with [¹²⁵I] microscale standards (PerkinElmer/NEN). This protocol has been successfully employed for both rat and monkey brain tissue (Sanchez et al., 1999; Skelton et al., 2000). Representative brain sections are shown in Figure 1C,D.

CRF receptor nonspecific binding

A third set of adjacent slides were processed for nonspecific binding for both the CRFR₁ and CRFR₂. The tracer buffer contained 0.2 nM [¹²⁵I-Tyr⁰]-sauvagine plus 1 mM cold sauvagine (American Peptide, Sunnyvale, CA). Slides were apposed to Kodak MR film for 85 hours. This protocol for nonspecific binding has been successfully employed for both rat and monkey brain tissue (Sanchez et al., 1999; Wigger et al., 2004). Representative brain sections are shown in Figure 1E,F.

Acetylcholinesterase (AChE) stain

Following CRFR₁ autoradiography and film development, slides were counterstained for acetylcholinesterase to better delineate the brain regions for image analysis. We modified the traditional AChE protocol to amplify AChE signal since the tissue had already undergone receptor autoradiography (Lim et al., 2004a). Briefly, slides were incubated for 5 hours in an enzymatic solution containing 0.0072% ethopropazine, 0.075% glycine, 0.05% cupric sulfate, 0.12% acetyl thiocholine iodide, 0.68% sodium acetate, pH 5.0. Slides were then rinsed in ddH₂O, developed for 30 minutes in a solution composed of 0.38% sodium sulfide at pH 7.8, rinsed again in dH₂O, and then exposed to a silver intensification solution (1% silver nitrate) for 10 minutes. Slides were rinsed in dH₂O, air-dried, dehydrated in ascending ethanols, cleared with Clearing Agent (Electron Microscopy Sciences, Fort Washington, PA), and coverslipped with DPX (Electron Microscopy Sciences). Images were compared with AChE-stained brain sections in rat and mouse brain atlases (Paxinos and Franklin, 2001; Paxinos and Watson, 1998).

Data analysis

Total CRF receptor binding, CRFR₂ binding, and nonspecific binding were quantified using AIS 6.0 software (Imaging Research, Ontario, Canada). AChE-stained sections were used to delineate the borders of brain regions of interest before sampling. Measurements were taken

bilaterally and averaged for each brain region across two or three sections. All optical density readings were automatically converted into decompositions per minute per milligram tissue (DPM/mg) based on a known set of [125 I] microscale standard values included on each film. Brain regions were quantified by an experimenter blind to the species and sex of the individuals.

Specific CRFR₂ binding values were obtained by subtracting nonspecific binding values from [125 I-Tyr⁰]-sauvagine binding in the presence of 1 μ M CP-154,526. Specific CRFR₁ binding values were calculated by subtracting nonspecific binding from the total [125 I-Tyr⁰]-sauvagine, and then subtracting CRFR₂ binding. Only values above two standard deviations from the nonspecific binding values were considered detectable.

Statistics

Due to the large number of regions sampled, brain regions were regrouped into anatomically or functionally similar groups which were comprised of 7–10 subregions. The regional groupings are detailed in Tables 1–4. Each group was analyzed using a two-between, one-within subject repeated measures ANOVA, where species and sex were the between-subject factors, and brain region the within-subject factor. Significant interactions were followed by tests for simple effects (univariate *t*-tests) as previously described (Nair et al., 1999; Stevens, 1996). The alpha level was set at 0.05 divided by 8 (0.00625), to control for multiple comparisons. Therefore, a *P*-value less than or equal to 0.05 after this modified Bonferroni adjustment was considered significant.

Photomicrograph production details

Digital images were obtained from film autoradiograms using a B-95 Northern Light light box (Imaging Research) and a SPOT camera (Diagnostic Instruments, Sterling Heights, MI) connected to a computer through the RT SPOT power supply. Digital images obtained from microscope slides were similarly taken using the SPOT camera setup. Images were then imported into Adobe PhotoShop 7.0 (Adobe Systems, San Jose, CA), cropped to the correct size, and minimally adjusted for brightness and contrast in order to clarify the scientific point of interest. All figure text, arrows, and scale bars were added using Adobe Illustrator 10.0.

RESULTS

Distribution of CRFR₁ in meadow and prairie voles

Mean CRFR₁ values (DPM/mg) from several brain regions in both male and female promiscuous meadow and monogamous prairie voles are shown in Table 1. Selected CRFR₁ meadow and prairie vole species comparisons are shown in Figure 2.

There were no significant species or sex differences in CRFR₁ binding in the cortex, septal-hippocampal system, or extended amygdala. In cortical regions, prairie and meadow voles expressed high levels of CRFR₁, as has been reported for rat, mouse, and nonhuman primate (Aguilera et al., 1987; De Souza et al., 1985; Potter et al., 1994; Primus et al., 1997; Sanchez et al., 1999; Van Pett et al., 2000). Prairie and meadow voles demonstrated only moderate CRFR₁ binding in the septo-hippocampal system and a minimal level of binding in the BNST and amygdalar subregions. This is in contrast to other rodent and primate species which express higher levels of the receptor in these regions (Aguilera et al., 1987; De Souza et al., 1985; Potter et al., 1994; Primus et al., 1997; Sanchez et al., 1999; Van Pett et al., 2000).

Striatum and habenula—A significant species by brain region interaction ($F = 25.1$, $P < 0.05$) was found in the striatal and habenular regions, such that meadow voles showed significantly higher CRFR₁ binding in the spNAcc, CPUv, and shNAcc than prairie voles (P

< 0.01 for all regions) (Fig. 2E,F) ($P < 0.01$). In contrast, prairie voles showed approximately 3-fold higher density of CRFR₁ binding in the mHab than meadow voles ($P < 0.01$) (Fig. 2H,I).

Olfactory and hindbrain—Both prairie and meadow voles showed CRFR₁ labeling in these olfactory and hindbrain regions. A significant species by brain region interaction ($F = 10.5$, $P < 0.01$) followed by tests for simple effects revealed that prairie voles showed less labeling in both the OB (Fig. 2B,C) and SColl (Fig. 2K,L) as compared to meadow voles ($P < 0.05$).

While it may appear that prairie voles might have lower overall R1 binding or lower R1 affinity for [¹²⁵I-Tyr⁰]-sauvagine than meadow voles, there were several brain regions where prairie voles have higher R1 binding density than meadow voles, such as in the medial habenula. Therefore, we doubt the differences that we observe are due to lower overall binding or affinity.

Distribution of CRFR₂ in prairie and meadow voles

Mean values for CRFR₂ binding (DPM/mg) from several brain regions in both male and female meadow and prairie voles are shown in Table 2. Selected CRFR₂ species comparisons are shown in Figure 3.

No species or sex differences were found in the cortex, extended amygdala, olfactory, and hindbrain nuclei. There was a nonsignificant trend for higher binding in the principal motor nucleus of 5 in prairie versus meadow voles (Fig. 3K,L). Among the hindbrain nuclei, the dorsal raphe had the highest levels of binding, while in cortical, extended amygdalar, olfactory, and other hindbrain nuclei, CRFR₂ binding was minimal in both species. These findings are generally consistent with other rodent and nonhuman primate studies, although these species tend to show much higher CRFR₂ binding in the extended amygdala (Potter et al., 1994; Sanchez et al., 1999; Van Pett et al., 2000).

Septal-hippocampal regions—As reported for other rodent species (but not in primate) (Potter et al., 1994; Van Pett et al., 2001; Sanchez et al., 1999), dense CRFR₂ binding was observed in the lateral septum in meadow voles. However, unlike rat and mouse, meadow voles also showed dense binding in the hippocampal fields. Meadow voles showed significantly higher CRFR₂ binding in the dorsal caudal lateral septum (cLSD) than prairie voles ($P < 0.05$ following a significant species by brain region interaction ($F = 9.6$, $P < 0.01$) (Fig. 3E,F). In addition, meadow voles showed significantly higher CRFR₂ binding than prairie voles in all the hippocampal fields, with the most dramatic species differences in the CA1, and to a lesser extent the CA2 and CA3 fields ($P < 0.001$, $P < 0.01$, $P < 0.01$, respectively) (Fig. 3H,I).

Striatum and habenula—Unlike other rodent and primate species, monogamous prairie voles very strongly express CRFR₂ binding in the striatum. A significant species by brain regions interaction ($F = 45.8$, $P < 0.001$) indicated that prairie voles had significantly higher CRFR₂ binding than meadow voles in the spNAcc, and CPUv ($P < 0.01$ in all cases) (Fig. 3B,C).

It is interesting again to note that there are several regions where prairie voles exceed meadow voles, and vice versa. Therefore, it is unlikely that global species differences in CRFR₂ affinity exist. In fact, no sex or species differences exist in the nonspecific binding values obtained when [¹²⁵I-Tyr⁰]-sauvagine is incubated with the cold ligand (as seen previously in Fig. 1).

Distribution of CRFR₁ in pine and montane voles

Mean values (DPM/mg) for CRFR₁ from several brain regions in both male and female promiscuous montane and monogamous pine voles are shown in Table 3. Selected CRFR₁ montane and pine vole species comparisons are shown in Figure 4.

There were no differences between pine and montane voles in CRFR₁ binding in the septal-hippocampal system or extended amygdala. CRFR₁ binding in these regions was comparable to prairie and meadow voles.

Cortical regions—Like meadow and prairie voles, montane and pine voles also showed moderate to dense CRFR₁ binding throughout the cortex. However, in contrast to the meadow/prairie comparison, an overall significant species by brain region interaction ($F = 3.4$, $P < 0.005$) followed by simple effects tests indicated that montane voles demonstrated greater binding in the prefrontal cortex (PFCtx), insular cortex (IFCtx), orbitofrontal cortex (OFCtx), cingulate cortices (CgCtx1 and CgCtx2), and the retrosplenial cortex (RSCtx) ($P < 0.05$).

Striatum and habenula—The striatal and habenular regions demonstrated a significant species by brain region interaction ($F = 18.3$, $P < 0.001$). Simple effects tests revealed that montane voles demonstrated greater CRFR₁ binding in the shNAcc and CPu relative to monogamous pine voles (Fig. 4E,F). There were striking species differences in the laterodorsal thalamus (ldThal), a novel brain region which did not show CRFR₁ binding in meadow or prairie voles. Montane voles had very dense CRFR₁ binding in this region, while pine voles were virtually devoid of binding ($P < 0.001$) (Fig. 4H,I).

Olfactory and hindbrain—Montane voles had significantly higher CRFR₁ binding in the olfactory bulb and the superior colliculus relative to pine voles ($F = 3.0$, $P < 0.05$, overall brain by species interaction) (Fig. 4B,C and 4K,L, respectively). In addition, montane voles also had higher CRFR₁ binding in the locus coeruleus and pontine dorsal tegmental nucleus ($P < 0.05$). There were no species differences observed in the other hindbrain regions, including the PAG, DR, Pr5, and CBg.

Distribution of CRFR₂ in montane and pine voles

Mean values (DPM/mg) for CRFR₂ from several brain regions in both male and female montane and pine voles are shown in Table 4. Selected CRFR₂ species comparisons are shown in Figure 5. Data will be discussed primarily in relation to the species differences found between prairie and meadow voles.

Cortical regions—Consistent with cortical regions in meadow and prairie voles, minimal to moderate CRFR₂ binding was observed in montane and pine voles. However, unlike meadow and prairie voles, who showed no significant species differences in cortex, pine voles showed significantly higher CRFR₂ than montane voles in the IFCtx, CgCtx1, CgCtx2, and CgCtx3 ($F = 6.7$, $P < 0.05$) (Fig. 5B,C, 5E,F).

Septal-hippocampal regions—Like meadow and prairie voles, there were also significant differences in CRFR₂ in the lateral septum and hippocampus between montane and pine voles. However, whereas meadow voles had higher CRFR₂ in these regions than prairie voles, the opposite was observed for pine and montane voles. Pine voles had nearly a 4-fold increase in lateral septum CRFR₂ binding than montane voles, as well as significantly more CRFR₂ in the CA1 and CA3 fields of the hippocampus ($F = 22.7$, $P < 0.001$) (Fig. 5E,F and 5H,I, respectively).

Extended amygdala—Similar to that observed in the meadow and prairie vole comparison, montane and pine voles expressed weak and diffuse CRFR₂ binding throughout the bed nucleus of the stria terminalis and amygdala nuclei. No significant species differences were observed in the mBnST, lBnST, cBnST, or any of the subnuclei of the amygdala ($P > 0.05$).

Striatum and habenula—Montane and pine voles showed a significant species by brain region interaction in these subregions ($F = 25.3$, $P < 0.001$). Pine voles had significantly higher CRFR₂ binding in the septal pole of the nucleus accumbens (spNAcc) and caudate-putamen than montane voles (Fig. 5B,C). Like meadow and prairie voles, there were no significant species differences in CRFR₂ binding in the shNAcc or mHab ($P > 0.05$), although the lHab was marginally significantly different ($P < 0.05$). CRFR₂ levels in the laterodorsal thalamus (ldThal) were significantly higher in montane voles compared to pine voles ($P < 0.001$) (Fig. 5H,I).

Olfactory and hindbrain—Unlike the meadow and prairie comparison, there were some olfactory and hind-brain regions that significantly differed between montane and pine voles ($F = 15.3$, $P < 0.001$). CRFR₂ levels in the olfactory bulb were marginally significantly elevated in pine voles compared to montane voles ($P < 0.05$). CRFR₂ levels in the dorsal raphe and cerebellum were also significantly elevated in pine voles compared to montane voles ($P < 0.01$) (Fig. 5K,L). There were no differences observed in the other hindbrain regions analyzed.

Like the meadow and prairie vole comparison, there are several regions for both CRFR₁ and CRFR₂ where montane voles exceed pine voles and vice versa. Therefore, it is unlikely that global species differences in CRF receptor affinity for the ligand exist between pine and montane voles.

Sex differences in CRFR₁ and CRFR₂ distribution

No sex differences have been previously reported in CRFR₁ or CRFR₂ in any other species mapping studies (Aguilera et al., 1987; De Souza et al., 1985; Potter et al., 1994; Primus et al., 1997; Sanchez et al., 1999; Van Pett et al., 2000). However, in vole species, large sex differences in CRFR₂ binding were detected in the medial caudal subdivision of the BnST (cBnST), also known as the encapsulated or posteromedial subdivision, which expresses CRFR₂ in both rats and mice (Van Pett et al., 2000). In the prairie and meadow vole comparison, males of both species displayed significantly higher CRFR₂ binding than females of both species, approximating a 2–3-fold increase ($P < 0.01$, post-hoc Bonferroni adjusted *t*-test) (Fig. 6). In the pine and montane vole comparison, males of both species also showed significantly higher CRFR₂ binding than females of both species, although to a somewhat lesser extent than in the prairie and meadow comparison ($P < 0.05$) (Fig. 7).

Whole brain CRFR₁ and CRFR₂ binding did not significantly differ between the sexes, suggesting that sex differences in receptor expression were in fact specific to particular brain regions.

DISCUSSION

Given the remarkable species differences in social structure between the monogamous prairie and pine vole and promiscuous meadow and montane vole, species differences likely exist in the brain that might explain the proximate mechanisms of behavior. Past literature has focused predominantly on the neuropeptide systems oxytocin and vasopressin to explain the species differences in social behavior; however, recent studies have shown that corticosterone and CRF modulate partner preference in prairie voles as well (DeVries et al., 1995, 1996, 2002). Our results show that monogamous versus promiscuous vole species pairs in fact have dramatically different CRFR₁ and CRFR₂ distributions in the brain. These data support the hypothesis that CRF plays a role in the monogamous-typical behaviors of the prairie vole, insofar as central CRF release would potentially activate different neural circuits, depending on where CRFR₁ and CRFR₂ are expressed in the brain. For example, it is possible that CRFR₁ and CRFR₂ patterns may fall within specific neural circuits that elicit affiliative, monogamous social behavior in prairie and pine voles, whereas CRFR₁ and CRFR₂ may be absent in these neural

circuits in promiscuous species. Identifying these differences may generate further hypotheses as to which brain regions are specifically involved in CRF-induced pair bond formation.

Vole species differences in CRFR₁ brain distribution

Differences in CRFR₁ distribution between meadow and prairie voles were observed throughout several brain regions. Most notably, large differences appeared in limbic areas such as the shell of the nucleus accumbens (shNAcc) and the medial habenula, and more subtle differences appeared in the olfactory bulb (OB) and the superior colliculus (SColl). Differences in CRFR₁ distribution between montane and pine voles were also observed throughout the brain, including cortical regions, the striatum (including shNAcc), and similarly subtle differences in the OB and SColl. These global patterns of CRFR₁ in the shNAcc, OB, and SColl, which are increased in promiscuous meadow and montane voles compared to monogamous prairie and pine voles, suggest that these patterns could underlie a neural circuit contributing to monogamous social behavior.

Vole species differences in CRFR₂ brain distribution

We observed dramatic species differences between meadow and prairie voles in CRFR₂ distribution in several brain regions, including the striatum (caudate and NAcc), the lateral septum, and CA1 field of the hippocampus. However, not all these species differences were replicated in our second comparison between montane and pine voles. In fact, some of the species differences went in the opposite direction from predicted. For example, in both the lateral septum and hippocampus, meadow voles had more CRFR₂ than prairie voles, yet pine voles had more CRFR₂ than montane voles in these regions. But in the striatum, and especially in the septal pole of the NAcc, both monogamous vole species had significantly higher CRFR₂ than the two promiscuous vole species. The significance of CRFR₂ in the NAcc in pair bond formation in monogamous species remains to be determined. It is interesting to note that the NAcc has already been implicated in regulating partner preference formation in prairie voles: Activation of oxytocin receptors and dopamine D2 receptors in the NAcc are both necessary and sufficient for pair bond formation in prairie voles (Aragona et al., 2003; Gingrich et al., 2000; Liu and Wang, 2003; Young et al., 2001). It is possible that CRFR₂ in the NAcc may also interact with existing oxytocin and dopaminergic systems to facilitate pair bond formation.

Vole sex differences in the encapsulated BnST

Sex differences in CRFR₂ binding in the medial caudal bed nucleus of the stria terminalis (cBnST), or encapsulated subdivision, were observed in all four vole species. Males of all species had significantly higher CRFR₂ binding in the cBnST than females of all species. This is a particularly interesting finding in light of the fact that no other experiments in rats, mice, or nonhuman primates have reported sex differences in CRFR₁ or CRFR₂ in any brain region (Aguilera et al., 1987; De Souza et al., 1985; Potter et al., 1994; Primus et al., 1997; Sanchez et al., 1999; Van Pett et al., 2000). The cBnST is a prominent brain region that exhibits sex differences in gonadal steroid systems such as estrogen and androgen receptors and aromatase expression in rat (Herbison, 1995; Herbison and Fenelon, 1995; Wagner and Morrell, 1997). A recent study suggested that CRFR₂ may also play a role in behavioral sex differences: Female CRFR₂ knockout mice showed opposite behavioral responses to swim stress compared to male CRFR₂ knockout mice (Bale and Vale, 2003). Interestingly, past studies in prairie voles have shown that the effects of exogenous corticosterone on pair bond formation are also sexually dichotomous, analogous to the findings in CRFR₂ knockout mice (DeVries et al., 1996). Since we observed sex differences in BnST CRFR₂ binding in voles, it is possible that these brain differences might underlie the sexual dichotomy in pair bond formation in prairie voles. For example, canonical HPA axis feedback could potentially modulate central CRF release onto

CRFR₂ in the cBnST, which could then lead to inhibition or facilitation of partner preference, depending on CRFR₂ density in the cBnST. The BnST has been implicated in depressive-like behaviors, and there is also a prominent gender difference in the epidemiology of depression in humans (Erb et al., 2001; Stout et al., 2000; Young, 1998). Perhaps vole species could represent a novel animal model in which sex differences in social behavior could be further studied.

CRF and social behavior

Despite the abundance of studies on CRF and stress/anxiety, relatively few studies in the literature have examined the role of the CRF system in social behavior. One study examining the behavioral effects of chronic CRF infusion on rhesus monkeys housed either singly or in groups found increased depressive-like behaviors only in the socially housed monkeys (Strome et al., 2002). Another recent study found lower concentrations of CRF in the cerebrospinal fluid of bonnet macaques, which are gregarious and affiliative, than pigtail macaques, which are more socially distant (Rosenblum et al., 2002).

How could CRF be acting to modulate social behavior? In a way, social behavior overlaps with stress and anxiety, especially in behaviors involving social support or coping with social isolation. Previous research in prairie voles has shown that plasma corticosterone levels elevate during social separation from the partner, and reunion with the familiar partner is followed by a return to baseline (Carter et al., 1997). In naïve prairie voles, both male and female prairie voles experience a reduction in corticosterone levels within an hour of being paired with an animal of the opposite sex, although males tend to show a greater reduction than females (DeVries et al., 1997). A recent preliminary study using prairie voles showed that recently separated pair-bonded prairie voles exhibited more “depressive-like” symptoms in the forced swim test than their sibling-separated counterparts (Bosch et al., 2004). These data suggest that CRF systems do have a role in modulating social behavior, and this is especially apparent in an animal model whose affiliative social behavior is its defining feature.

Evolutionary implications

Because it is currently unknown whether vole species have similar CRFR₁ and CRFR₂ to those which have been identified in rat, mouse, monkey, and human, it is possible that there could be other species differences that we have not reported. For example, it is unknown what the affinity of the endogenous ligands for these putative CRFR₁ and CRFR₂ is in voles. Thus, our results should be interpreted accordingly. However, our results do suggest that voles do have CRFR₁- and CRFR₂-like receptors, since [¹²⁵I-Tyr⁰]-sauvagine bound to the tissue in all four species, and cold sauvagine competed the [¹²⁵I-Tyr⁰]-sauvagine completely off. Furthermore, there are some core areas which are highly conserved across all species examined, such as the choroid plexus, cortex, raphe, cerebellum, and olfactory bulb.

Given the assumption that vole species have similar CRFR₁ and CRFR₂ as characterized in other species, a broader question is: What is the significance of these species similarities and differences in elucidating CRF receptor function? When one compares CRF distribution patterns in the four vole species to those in rat and nonhuman primate, CRF patterns appear to segregate into three different classes: 1) those that are conserved across mammalian species, including rat, monkey, and the four vole species; 2) those that are phylogenetically plastic across the six species, including within closely related vole species; and 3) those that segregate based on social organization. This conceptual framework, summarized in Tables 5 and 6, might be used as a general heuristic in understanding the functional and evolutionary significance of different CRF systems.

CRFR₁ brain distribution across six species

Both similarities and differences in CRFR₁ distribution were observed throughout the brain across species (Table 5). Data are summarized from meta-analysis of past reports of CRFR₁ receptor binding in rat and rhesus macaque brains (Aguilera et al., 1987; Chalmers et al., 1995; De Souza, 1987; De Souza et al., 1985; Grigoriadis et al., 1995; Primus et al., 1997; Rominger et al., 1998; Sanchez et al., 1999; Steckler and Holsboer, 1999). The cortex, olfactory bulb, cerebellum, and superior colliculus appeared the most highly conserved in terms of similar relative CRFR₁ binding, and had strong to moderate binding across all six species. Therefore, cortical, olfactory, and cerebellar CRFR₁ may represent “classic,” or typical functions of CRFR₁ since the dense binding is so highly conserved across rodent and primate species.

CRFR₁ binding patterns in other regions appear to distinguish voles from rats and nonhuman primates, and hence may reflect the phylogenetic relatedness of the four vole species. For example, the lateral septum had moderate to dense CRFR₁ binding in all four vole species, but is devoid of CRFR₁ binding in rat and monkey. The hippocampus, amygdala, and periaqueductal gray demonstrate strong to moderate CRFR₁ binding in rat and monkey, but little to no binding in all four vole species. It is possible that CRFR₁ receptor function in these regions could play an important role in producing behaviors specific to vole species.

Some brain regions differ between species in a less predictable pattern: The habenula, laterodorsal thalamus, locus coeruleus, and dorsal raphe all show remarkable plasticity across species, but with no obvious pattern. Perhaps the functional significance of CRF receptors in these brain regions could reflect adaptation to a particular ecological niche, such as enhanced spatial memory or specific stress response to predation.

Additional comparisons can be made between species with different social organizations. Since prairie and pine voles are the only species with monogamous social organization out of the six species examined, one would predict that brain regions involved in monogamous behavior would yield CRFR₁ patterns common to just prairie and pine voles. For example, the olfactory bulb, superior colliculus, and shell of the NAcc fit this criterion, as they all have higher CRFR₁ binding in promiscuous vole species. It is possible that the olfactory bulb, superior colliculus, and NAcc comprise a CRF-relevant neural circuit that could relate to nonmonogamous behaviors. In support of this hypothesis, nonmonogamous rats do show minimal to moderate CRFR₁ expression in these regions as well, although it is difficult to draw a solid conclusion from this single observation.

CRFR₂ brain distribution across six species

Semiquantitative similarities and differences for CRFR₂ brain distribution across all four vole species, rat, and monkey are summarized in Table 6. Data were compiled from meta-analysis of prior reports of CRFR₂ receptor binding in rat and rhesus macaque (Aguilera et al., 1987; Chalmers et al., 1995; De Souza, 1987; De Souza et al., 1985; Grigoriadis et al., 1995; Primus et al., 1997; Rominger et al., 1998; Sanchez et al., 1999; Steckler and Holsboer, 1999). CRFR₂ binding in several brain regions was highly conserved across the six species, including the choroid plexus, dorsal raphe, olfactory bulb, and cortex. CRFR₂ functions in these regions could potentially represent prototypical CRFR₂ physiology since they are so highly conserved across these unrelated species.

Some brain regions are conserved only within the four vole species, such as the amygdala, periaqueductal gray, locus coeruleus, and cerebellum, which show minimal to moderate binding in voles, but are different in rat and monkey. Other brain regions are extraordinarily plastic in their CRFR₂ expression across species, such as the lateral septum, hippocampus,

laterodorsal thalamus, and superior colliculus. CRFR₂ in the lateral septum and hippocampus are particularly interesting in this regard because of their roles in modulating basic learning and memory systems (Bakshi et al., 2002; Radulovic et al., 1999). One would predict that if CRFR₂ in these regions were extremely critical for basic learning and memory, their patterns might appear more conserved across mammals. It is possible that such wide variation in CRFR₂ binding in these regions reflects species differences in adaptation to a particular ecological niche, which could require different specializations for spatial memory or fear learning of predators, for example.

Only one brain region cleanly segregates with social organization. CRFR₂ binding in the septal pole of the nucleus accumbens is much higher in monogamous vole species compared to promiscuous vole species. In support of this observation, CRFR₂ binding is similarly absent in the nonmonogamous rat (but was not reported in nonmonogamous rhesus macaque) (Potter et al., 1994; Primus et al., 1997; Sanchez et al., 1999). The significance of CRFR₂ in the NAcc in pair bond formation remains to be determined, but it would be interesting to compare CRFR₂ binding in the septal pole of the NAcc in additional monogamous-promiscuous species pairs beyond just vole species.

What are the evolutionary implications for this plasticity in neuropeptide systems in such closely related vole species, as well as across other species? Vasopressin, oxytocin, and CRF receptors all show tremendous variation in brain distribution across species (Barberis and Tribollet, 1996; Goodson and Bass, 2001). However, classical neurotransmitter systems such as dopamine receptors and serotonin transporter distributions are highly conserved within voles and other rodents, as well as cholinergic systems as measured by acetylcholinesterase staining (unpubl. data, Lim and Young). It appears that neuropeptide systems, which are typically more modulatory in nature and control social behaviors, are evolutionarily more plastic than these classical neurotransmitter systems. This tendency for rapid change in neuropeptide receptor distribution can in turn produce large variation in social behavior, which can then be shaped by natural selection depending on environment. Thus, neuropeptide receptor plasticity could be a critical substrate for the rapid evolution of social behavior within a species.

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Fig. 1.

Validation of CRF receptor autoradiography technique. **A:** Meadow vole brain section incubated with [¹²⁵I-Tyr⁰]-sauvagine, which labels both CRFR₁ and CRFR₂. **B:** Prairie vole brain section incubated with [¹²⁵I-Tyr⁰]-sauvagine. **C:** Meadow vole brain section incubated with [¹²⁵I-Tyr⁰]-sauvagine plus the CRFR₁ selective inhibitor CP-154,526, which ultimately labels only CRFR₂. **D:** Prairie vole brain section labeling for CRFR₂. **E:** Meadow vole brain section incubated with [¹²⁵I-Tyr⁰]-sauvagine plus cold sauvagine, ultimately showing the nonspecific binding. **F:** Prairie vole brain section showing nonspecific binding. Scale bar = 1 mm in F (applies to A–F).

Fig. 2. Total CRF receptor binding, highlighting differences in CRFR₁ between promiscuous meadow and monogamous prairie vole species. Meadow vole (**B**) and prairie vole (**C**) brain sections through the olfactory bulb. Meadow vole (**E**) and prairie vole (**F**) brain sections through the nucleus accumbens. Note the strikingly dense CRFR₁ binding in the shell of the NAcc in the meadow vole. Meadow vole (**H**) and prairie vole (**I**) brain sections showing the species difference in CRFR₁ binding in the medial habenula nucleus. Note that the species difference in CRFR₁ in the medial habenula is in the opposite direction as the species difference in the shell of the NAcc. Meadow vole (**K**) and prairie vole (**L**) hindbrain section highlighting the

superior colliculus. Acetylcholinesterase-stained adjacent sections are shown to the left of each panel. Scale bars = 1 mm in C (applies to A,B,C), L (applies to D–L).

Fig. 3. CRFR₂ binding in promiscuous meadow and monogamous prairie vole species. Meadow vole (**B**) and prairie vole (**C**) sections through the striatum. Meadow vole (**E**) and prairie vole (**F**) brain sections through the lateral septum. Note that the species difference in CRFR₂ in the lateral septum is in the opposite direction as the species difference in the striatum. Meadow vole (**H**) and prairie vole (**I**) brain sections through the hippocampus. Note the dense labeling in the CA1 fields. Meadow vole (**K**) and prairie vole (**L**) hindbrain sections through the principal nucleus of five. Acetylcholinesterase-stained adjacent sections are shown to the left of each panel. Scale bar = 1 mm in L (applies to A–L).

Fig. 4. Total CRF receptor binding, highlighting differences in CRFR₁ between promiscuous montane and monogamous pine vole species. Montane vole (**B**) and pine vole (**C**) brain sections through the olfactory bulb. Montane vole (**E**) and pine vole (**F**) brain sections through the nucleus accumbens. Note the strikingly dense CRFR₁ binding in the shell of the NAcc in the montane vole. Montane vole (**H**) and pine vole (**I**) brain sections showing the species difference in CRFR₁ binding in the laterodorsal thalamus. Montane vole (**K**) and pine vole (**L**) hindbrain section highlighting the superior colliculus. Acetylcholinesterase-stained adjacent sections are shown to the left of each panel. Scale bar = 1 mm in C (applies to A,B,C), L (applies to D-L).

Fig. 5. CRFR₂ binding in promiscuous montane and monogamous pine vole species. Montane vole (**B**) and pine vole (**C**) sections through the striatum. Note that the species difference in CRFR₂ in the septal pole of the NAcc is in the same direction as the meadow versus prairie comparison. Montane vole (**E**) and pine vole (**F**) brain sections through the lateral septum. Note that the species difference in CRFR₂ in the lateral septum is in the opposite direction as the meadow versus prairie comparison. Montane vole (**H**) and pine vole (**I**) brain sections through the hippocampus. Note the species differences in the CA1 fields and the laterodorsal thalamus. Montane vole (**K**) and pine vole (**L**) hindbrain sections through the dorsal raphe

nucleus. Acetylcholinesterase-stained adjacent sections are shown to the left of each panel. Scale bar = 1 mm in L (applies to A–L).

Fig. 6. Sex differences in CRFR₂ binding in the BnST in promiscuous meadow and monogamous prairie voles. **A:** Rat brain schematic of the medial caudal, or encapsulated, BnST and fornix (Paxinos and Watson, 1998). **B:** Meadow male. **C:** Prairie male. **D:** Acetylcholinesterase staining of an adjacent brain section through the BnST. **E:** Meadow female. **F:** Prairie female. Note the CRFR₂ binding in the choroid plexus (ChP) is roughly equivalent across sex and species. Scale bar = 1 mm in F (applies to A–F).

Fig. 7. Sex differences in CRFR₂ binding in the BnST in promiscuous montane and monogamous pine voles. **A:** Rat brain schematic of the medial caudal, or encapsulated, BnST and fornix (Paxinos and Watson, 1998). **(B)** Montane male. **(C)** Pine male. **(D)** Acetylcholinesterase staining of an adjacent brain section through the BnST. **(E)** Montane female. **(F)** Pine female. Note the CRFR₂ binding in the choroid plexus (ChP) is roughly equivalent across sex and species. Scale bar = 1 mm in F (applies to A-F).

TABLE 1
Comparison of Prairie and Meadow Voles: CRFR₁ Binding Using 125-I-Sauvagine and CRFR₂ Subtraction¹

Region	Abbreviation	Meadow Female Mean $\pm$ SEM	Meadow Male Mean $\pm$ SEM	Prairie Female Mean $\pm$ SEM	Prairie Male Mean $\pm$ SEM
Cortical					
Prefrontal	PFCtx	3748 $\pm$ 394	3472 $\pm$ 624	5021 $\pm$ 625	3434 $\pm$ 304
Insular	IFCtx	6041 $\pm$ 533	5204 $\pm$ 1150	6989 $\pm$ 819	4681 $\pm$ 446
Orbitofrontal	OFCtx	4432 $\pm$ 474	3867 $\pm$ 594	5193 $\pm$ 1022	2689 $\pm$ 193
Cingulate rostral	CgCtX1	4530 $\pm$ 333	3856 $\pm$ 639	4229 $\pm$ 435	3083 $\pm$ 256
Cingulate middle	CgCtX2	4313 $\pm$ 367	2721 $\pm$ 94	3912 $\pm$ 416	2475 $\pm$ 221
Cingulate caudal	CgCtX3	4022 $\pm$ 167	2576 $\pm$ 83	3688 $\pm$ 240	2653 $\pm$ 105
Retrosplenial	RSCtx	2523 $\pm$ 245	1684 $\pm$ 129	1838 $\pm$ 357	1684 $\pm$ 212
Septal-hippocampal					
Lateral septum	LS				
dorsal rostral	rLSD	2386 $\pm$ 683	1018 $\pm$ 345	4697 $\pm$ 1004	2295 $\pm$ 376
intermediata rostral	rLSI	1525 $\pm$ 470	801 $\pm$ 168	1998 $\pm$ 678	1599 $\pm$ 586
dorsal caudal	cLSD	2336 $\pm$ 803	1839 $\pm$ 313	6173 $\pm$ 1267	3309 $\pm$ 188
intermediata caudal	cLSI	1817 $\pm$ 642	1208 $\pm$ 636	1816 $\pm$ 695	1157 $\pm$ 593
Hippocampus	HC		ND	1024 $\pm$ 108	642 $\pm$ 174
CA1	CA1	717 $\pm$ 241	ND	811 $\pm$ 116	ND
CA2	CA2	779 $\pm$ 245	708 $\pm$ 242	929 $\pm$ 240	773 $\pm$ 205
CA3	CA3	923 $\pm$ 113	580 $\pm$ 228	723 $\pm$ 201	655 $\pm$ 292
dentate gyrus	DG	611 $\pm$ 296			
Extended amygdala					
Bed nucleus of the stria terminalis	BnST				
medial	mBnST	1349 $\pm$ 140	939 $\pm$ 132	1249 $\pm$ 209	600 $\pm$ 152
lateral	lBnST	1039 $\pm$ 55	ND	860 $\pm$ 113	677 $\pm$ 130
caudal	cBnST	705 $\pm$ 528	672 $\pm$ 532	957 $\pm$ 322	693 $\pm$ 281
Amygdala	Amyg				
central	CeA	667 $\pm$ 192	ND	879 $\pm$ 216	697 $\pm$ 275
basolateral rostral	rBLA	1558 $\pm$ 157	1002 $\pm$ 257	1126 $\pm$ 227	1143 $\pm$ 422
medial rostral	rMeA	2095 $\pm$ 382	1771 $\pm$ 243	1935 $\pm$ 254	1430 $\pm$ 322
cortical rostral	rCoA	2778 $\pm$ 442	3010 $\pm$ 374	3043 $\pm$ 196	2382 $\pm$ 415
basolateral caudal	cBLA	1358 $\pm$ 211	1392 $\pm$ 256	1413 $\pm$ 330	931 $\pm$ 273
medial caudal	cMeA	2335 $\pm$ 327	2778 $\pm$ 666	1443 $\pm$ 169	1306 $\pm$ 209
cortical caudal	cCoA	3958 $\pm$ 632	4127 $\pm$ 921	3360 $\pm$ 400	2472 $\pm$ 297
Striatum and habenula					
Nucleus accumbens	NAcc				
shell	shNAcc	6260 $\pm$ 740	3882 $\pm$ 1248	1145 $\pm$ 218	1109 $\pm$ 56
septal pole	spNAcc	1697 $\pm$ 164	1967 $\pm$ 256	ND	637 $\pm$ 409
Caudate-putamen	CPu				
dorsal	CPuD	1947 $\pm$ 184	1566 $\pm$ 430	1009 $\pm$ 277	790 $\pm$ 164
ventral	CPuV	1905 $\pm$ 113	1758 $\pm$ 225	983 $\pm$ 234	836 $\pm$ 213
Habenula					
medial	mHab	2172 $\pm$ 423	2078 $\pm$ 261	6926 $\pm$ 1182	5478 $\pm$ 1201
lateral	lHab	903 $\pm$ 294	545 $\pm$ 98	760 $\pm$ 139	553 $\pm$ 253
Thalamus					
laterodorsal	ldThal	ND	ND	ND	ND
Olfactory and hindbrain					
Olfactory bulb					
external plexiform	OB	15474 $\pm$ 774	17132 $\pm$ 2022	9831 $\pm$ 1498	9447 $\pm$ 2536
Superior colliculus	SColl	12600 $\pm$ 1766	10199 $\pm$ 2120	7297 $\pm$ 226	5638 $\pm$ 709
Periaqueductal gray	PAG	1512 $\pm$ 146	1196 $\pm$ 64	1362 $\pm$ 206	1147 $\pm$ 239
Dorsal raphe	DR	497 $\pm$ 281	ND	542 $\pm$ 501	847 $\pm$ 523
Locus coeruleus	LC	1621 $\pm$ 126	1483 $\pm$ 286	1835 $\pm$ 398	1536 $\pm$ 150

Region	Abbreviation	Meadow Female Mean $\pm$ SEM	Meadow Male Mean $\pm$ SEM	Prairie Female Mean $\pm$ SEM	Prairie Male Mean $\pm$ SEM
Pontine dorsal tegmental nucleus	PDTg	2063 $\pm$ 438	1763 $\pm$ 430	2057 $\pm$ 408	1433 $\pm$ 57
Principal motor nucleus of 5	Pr5	1509 $\pm$ 198	1060 $\pm$ 485	1173 $\pm$ 425	724 $\pm$ 306
Cerebellum granular layer	CBg	4611 $\pm$ 633	3348 $\pm$ 296	4650 $\pm$ 1025	3274 $\pm$ 355

¹Values represent mean DPM/mg tissue from n = 4 per group.

ND, not detected (<2 S.D. nonspecific binding);

* $P < 0.05$ species effect;

** $P < 0.01$ species effect;

[†] $P < 0.05$ sex effect.

TABLE 2

Comparison of Prairie and Meadow Voles: CRFR₂ Binding Using 125-I-Sauvagine and CP-154,526^I

Region	Abbreviation	Meadow Female Mean ± SEM	Meadow Male Mean ± SEM	Prairie Female Mean ± SEM	Prairie Male Mean ± SEM
Cortical					
Prefrontal	PFctx	3117 ± 83	3180 ± 112	2957 ± 78	2862 ± 123
Insular	IFctx	2937 ± 79	2906 ± 74	2906 ± 57	2884 ± 121
Orbitofrontal	OFCtx	2845 ± 125	2805 ± 79	2809 ± 30	2828 ± 77
Cingulate rostral	CgCtx1	3331 ± 150	3195 ± 153	3000 ± 67	2924 ± 66
Cingulate middle	CgCtx2	3066 ± 121	2905 ± 206	2842 ± 81	2955 ± 81
Cingulate caudal	CgCtx3	3007 ± 34	2918 ± 153	2832 ± 60	2858 ± 110
Retrosplanial	RSCtx	2846 ± 170	3305 ± 321	2660 ± 89	2768 ± 186
Septal-hippocampal					
Lateral septum	LS				
dorsal rostral	rLSD	5974 ± 475	6116 ± 572	4449 ± 298	4211 ± 579
intermediata rostral	rLSI	8306 ± 912	6662 ± 552	5830 ± 649	5583 ± 1138
dorsal caudal	cLSD **	6896 ± 604	6776 ± 602	4444 ± 182	4274 ± 485
intermediata caudal	cLSI	12374 ± 1601	9357 ± 747	7888 ± 1213	7635 ± 2000
Hippocampus	HC				
CA1	CA1 **	8534 ± 630	10404 ± 808	3598 ± 156	3978 ± 268
CA2	CA2 **	3727 ± 186	3763 ± 300	2830 ± 53	2832 ± 246
CA3	CA3 **	3332 ± 136	3339 ± 255	2656 ± 61	2735 ± 159
dentate gyrus	DG	4826 ± 727	4571 ± 494	2924 ± 88	2951 ± 166
Extended amygdala					
Bed nucleus of the stria terminalis	BnST				
medial	mBnST	2638 ± 96	2650 ± 122	2437 ± 38	2559 ± 73
lateral	lBnST	2503 ± 45	2503 ± 98	2442 ± 36	2501 ± 84
caudal	cBnST †	4154 ± 302	14671 ± 861	2563 ± 109	6214 ± 541
Amygdala	Amyg				
central	CeA	2412 ± 78	2504 ± 105	2410 ± 91	2514 ± 84
basolateral rostral	rBLA	2846 ± 84	2916 ± 118	3379 ± 243	3050 ± 161
medial rostral	rMeA	2613 ± 40	2724 ± 121	2960 ± 171	3091 ± 197
cortical rostral	rCoA	2988 ± 107	3117 ± 122	2797 ± 129	2839 ± 68
basolateral caudal	cBLA	2817 ± 61	2679 ± 91	3154 ± 248	2982 ± 159
medial caudal	cMeA	2722 ± 105	3015 ± 197	3150 ± 155	3250 ± 257
cortical caudal	cCoA	3144 ± 104	3240 ± 194	2630 ± 57	2746 ± 133
Striatum and habenula					
Nucleus accumbens	NAcc				
shell	shNAcc	2823 ± 60	2804 ± 176	2922 ± 132	2822 ± 90
septal pole	spNAcc **	3258 ± 210	2969 ± 228	6981 ± 641	6185 ± 703
Caudate-putamen	CPu				
dorsal	CPuD	2503 ± 62	2381 ± 111	2594 ± 153	2700 ± 136
ventral	CPuV **	2812 ± 51	2572 ± 145	4738 ± 520	4740 ± 421
Habenula					
medial	mHab	2704 ± 20	2818 ± 131	2716 ± 47	2611 ± 131
lateral	lHab	2645 ± 40	2683 ± 145	2469 ± 67	2477 ± 97
Thalamus					
laterodorsal	ldThal	ND	ND	ND	ND
Olfactory and hindbrain					
Olfactory bulb					
external plexiform	OB	2909 ± 97	3040 ± 45	3060 ± 30	3053 ± 191
Superior colliculus	SColl	2938 ± 129	3116 ± 174	2725 ± 77	2799 ± 149
Periaqueductal gray	PAG	2482 ± 51	2548 ± 80	2407 ± 69	2408 ± 166
Dorsal raphe	DR	7047 ± 740	8852 ± 981	8694 ± 1672	7250 ± 1372
Locus coeruleus	LC	2843 ± 138	2591 ± 100	2989 ± 189	2591 ± 181
Pontine dorsal tegmental nucleus	PDTg	2451 ± 32	2473 ± 106	3154 ± 203	2950 ± 223
Principal motor nucleus of 5	Pr5	3791 ± 160	3499 ± 237	4959 ± 470	5433 ± 952
Cerebellum granular layer	CBg	2689 ± 78	2751 ± 147	2807 ± 125	2631 ± 127

^I Values represent mean DPM/mg tissue from n = 4 per group.

ND, not detected (<2 S.D. nonspecific binding);

* *P* < 0.05 species effect;** *P* < 0.01 species effect;

[†] $P < 0.05$ sex effect.

TABLE 3
Comparison of Pine and Montane Voles: CRFR₁ Binding Using 125-I-Sauvagine and CRFR₂ Subtraction

Region	Abbreviation	Meadow Female Mean $\pm$ SEM	Meadow Male Mean $\pm$ SEM	Prairie Female Mean $\pm$ SEM	Prairie Male Mean $\pm$ SEM
Cortical					
Prefrontal	PFCtx ^{**}	1944 $\pm$ 345	1951 $\pm$ 124	777 $\pm$ 364	974 $\pm$ 184
Insular	IFCtx ^{**}	2839 $\pm$ 572	2904 $\pm$ 252	1246 $\pm$ 362	1165 $\pm$ 77
Orbitofrontal	OFCtx [*]	2873 $\pm$ 324	2813 $\pm$ 218	1261 $\pm$ 519	1757 $\pm$ 372
Cingulate rostral	CgCux1 ^{**}	1943 $\pm$ 119	1838 $\pm$ 225	980 $\pm$ 217	1145 $\pm$ 162
Cingulate middle	CgCux2 ^{**}	1553 $\pm$ 122	1700 $\pm$ 132	837 $\pm$ 307	959 $\pm$ 53
Cingulate caudal	CgCux3 ^{**}	1395 $\pm$ 59	1733 $\pm$ 227	711 $\pm$ 297	1137 $\pm$ 303
Retrospinal	RSCtx ^{**}	1681 $\pm$ 339	1213 $\pm$ 159	ND	499 $\pm$ 151
Septal-hippocampal					
Lateral septum	LS	3445 $\pm$ 895	4009 $\pm$ 861	3149 $\pm$ 2178	1949 $\pm$ 764
dorsal rostral	rLSD	3845 $\pm$ 1191	5609 $\pm$ 1001	5427 $\pm$ 4015	4297 $\pm$ 1540
intermediata rostral	rLSI	4821 $\pm$ 960	4470 $\pm$ 456	8864 $\pm$ 5168	7790 $\pm$ 2827
dorsal caudal	cLSD	6817 $\pm$ 1208	6657 $\pm$ 338	4054 $\pm$ 2508	8395 $\pm$ 3563
intermediata caudal	HC				
Hippocampus	CA1	651 $\pm$ 65	527 $\pm$ 44	ND	573 $\pm$ 50
	CA2	549 $\pm$ 120	533 $\pm$ 69	ND	465 $\pm$ 45
	CA3	657 $\pm$ 104	766 $\pm$ 102	ND	538 $\pm$ 85
dentate gyrus	DG	547 $\pm$ 144	775 $\pm$ 27	ND	ND
Extended amygdala					
Bed nucleus of the stria terminalis	BnST	835 $\pm$ 185	907 $\pm$ 331	836 $\pm$ 326	1247 $\pm$ 601
medial	mBnST	1582 $\pm$ 378	ND	ND	453 $\pm$ 199
lateral	lBnST	3863 $\pm$ 2189	2206 $\pm$ 556	3082 $\pm$ 1761	4059 $\pm$ 507
caudal	cBnST				
Amygdala	Amyg				
central	CeA	691 $\pm$ 135	740 $\pm$ 160	672 $\pm$ 179	980 $\pm$ 497
basolateral rostral	rBLA	589 $\pm$ 456	1340 $\pm$ 679	492 $\pm$ 86	813 $\pm$ 475
medial rostral	rMeA	1848 $\pm$ 738	1281 $\pm$ 318	952 $\pm$ 679	ND
cortical rostral	rCoA	1944 $\pm$ 279	2071 $\pm$ 380	970 $\pm$ 258	947 $\pm$ 178
basolateral caudal	cBLA	1221 $\pm$ 171	1430 $\pm$ 175	ND	498 $\pm$ 169
medial caudal	cMeA	2115 $\pm$ 544	1375 $\pm$ 43	858 $\pm$ 537	681 $\pm$ 208
cortical caudal	cCoA	1834 $\pm$ 169	2425 $\pm$ 296	799 $\pm$ 195	1148 $\pm$ 260
Striatum and habenula					
Nucleus accumbens	NAcc				
shell	shNAcc	4335 $\pm$ 608	4598 $\pm$ 719	977 $\pm$ 106	1043 $\pm$ 83
septal pole	spNAcc	1512 $\pm$ 348	1956 $\pm$ 198	2344 $\pm$ 944	3307 $\pm$ 729
Caudate-putamen	CPu				
dorsal	CPuD	2032 $\pm$ 468	2000 $\pm$ 435	967 $\pm$ 233	955 $\pm$ 181
ventral	CPuV	1353 $\pm$ 247	1612 $\pm$ 195	1137 $\pm$ 293	1247 $\pm$ 259
Habenula					
medial	mHab	781 $\pm$ 84	658 $\pm$ 75	ND	540 $\pm$ 163
lateral	lHab	730 $\pm$ 177	584 $\pm$ 124	883 $\pm$ 388	458 $\pm$ 170
Thalamus					
laterodorsal	ldThal	3380 $\pm$ 552	3384 $\pm$ 802	ND	563 $\pm$ 127
Olfactory and hindbrain					
Olfactory bulb					
external plexiform	OB [*]	11849 $\pm$ 1897	9461 $\pm$ 3408	5080 $\pm$ 1477	5787 $\pm$ 2041
Superior colliculus	SColl ^{***}	8202 $\pm$ 1908	7970 $\pm$ 945	1522 $\pm$ 279	1982 $\pm$ 319
Periaqueductal gray	PAG	752 $\pm$ 47	888 $\pm$ 50	599 $\pm$ 174	ND
Dorsal raphe	DR	3891 $\pm$ 1250	2991 $\pm$ 1109	1728 $\pm$ 1311	10495 $\pm$ 2245

Region	Abbreviation	Meadow Female Mean $\pm$ SEM	Meadow Male Mean $\pm$ SEM	Prairie Female Mean $\pm$ SEM	Prairie Male Mean $\pm$ SEM
Locus coeruleus	LC*	1365 $\pm$ 205	1226 $\pm$ 112	540 $\pm$ 389	484 $\pm$ 130
Pontine dorsal tegmental n.	PDTg*	796 $\pm$ 140	971 $\pm$ 170	ND	452 $\pm$ 188
Principal motor nucleus of 5	Pr5	1834 $\pm$ 276	1951 $\pm$ 630	2826 $\pm$ 879	1969 $\pm$ 742
Cerebellum granular layer	CBg	2222 $\pm$ 424	3399 $\pm$ 279	2396 $\pm$ 323	1966 $\pm$ 565

Values represent mean DPM/mg tissue from n = 4 per group.

ND, not detected (<2 S.D. nonspecific binding);

* $P < 0.05$ species effect;

** $P < 0.01$ species effect;

† $P < 0.05$ sex effect.

TABLE 4
Comparison of Pine and Montane Voles: CRFR₂ Binding Using 125-I-Sauvagine and CP-154,526¹

Region	Abbreviation	Meadow Female Mean $\pm$ SEM	Meadow Male Mean $\pm$ SEM	Prairie Female Mean $\pm$ SEM	Prairie Male Mean $\pm$ SEM
Cortical					
Prefrontal	PFCtx**	1390 $\pm$ 186	1033 $\pm$ 130	1913 $\pm$ 200	1567 $\pm$ 84
Insular	IFCtx	1181 $\pm$ 99	987 $\pm$ 74	1667 $\pm$ 123	1490 $\pm$ 109
Orbitofrontal	OFCtx *	1475 $\pm$ 130	1187 $\pm$ 127	2774 $\pm$ 530	1842 $\pm$ 143
Cingulate rostral	CgCtx1 *	1351 $\pm$ 136	1151 $\pm$ 55	1751 $\pm$ 154	1699 $\pm$ 123
Cingulate middle	CgCtx2 **	1286 $\pm$ 142	1118 $\pm$ 58	1772 $\pm$ 202	1820 $\pm$ 54
Cingulate caudal	CgCtx3 **	1277 $\pm$ 150	1137 $\pm$ 91	1871 $\pm$ 179	2023 $\pm$ 169
Retrospinal	RSCtx	1608 $\pm$ 200	1266 $\pm$ 57	1558 $\pm$ 150	1290 $\pm$ 94
Septal-hippocampal					
Lateral septum	LS				
dorsal rostral	RLSD **	9891 $\pm$ 2333	4869 $\pm$ 306	27167 $\pm$ 2369	30216 $\pm$ 4144
intermediata rostral	RLSI **	10054 $\pm$ 1655	7136 $\pm$ 654	23646 $\pm$ 2990	29935 $\pm$ 2470
dorsal caudal	CLSD **	8139 $\pm$ 1158	7593 $\pm$ 241	34474 $\pm$ 7817	32930 $\pm$ 3591
intermediata caudal	CLSI *	13629 $\pm$ 2659	8774 $\pm$ 453	24334 $\pm$ 5494	31306 $\pm$ 5716
Hippocampus	HC *				
CA1	CA1 *	1216 $\pm$ 119	1123 $\pm$ 127	2422 $\pm$ 398	1750 $\pm$ 139
CA2	CA2 *	1360 $\pm$ 51	1088 $\pm$ 106	1784 $\pm$ 389	1492 $\pm$ 102
CA3	CA3 **	1110 $\pm$ 49	1022 $\pm$ 104	1596 $\pm$ 117	1613 $\pm$ 153
dentate gyrus	DG	1401 $\pm$ 50	1130 $\pm$ 27	1497 $\pm$ 133	1481 $\pm$ 95
Extended amygdala					
Bed nucleus of the stria terminalis	BnST				
medial	mBnST	1945 $\pm$ 236	1603 $\pm$ 166	2508 $\pm$ 384	2675 $\pm$ 343
lateral	lBnST	1552 $\pm$ 426	1916 $\pm$ 247	2422 $\pm$ 313	1775 $\pm$ 239
caudal	cBnST†	2954 $\pm$ 1195	7088 $\pm$ 2378	5375 $\pm$ 1914	8536 $\pm$ 609
Amygdala	Amyg				
central	CeA	1043 $\pm$ 52	1017 $\pm$ 133	1169 $\pm$ 116	1143 $\pm$ 113
basolateral rostral	rBLA	2316 $\pm$ 393	1675 $\pm$ 452	1292 $\pm$ 172	1199 $\pm$ 148
medial rostral	mMeA	1442 $\pm$ 324	1438 $\pm$ 347	2264 $\pm$ 429	2927 $\pm$ 917
cortical rostral	rCoA	1470 $\pm$ 79	1242 $\pm$ 124	1504 $\pm$ 235	1702 $\pm$ 224
basolateral caudal	cBLA	1230 $\pm$ 101	1323 $\pm$ 107	1339 $\pm$ 181	1300 $\pm$ 93
medial caudal	cMeA	1168 $\pm$ 84	1318 $\pm$ 90	2237 $\pm$ 219	1485 $\pm$ 123
cortical caudal	cCoA	1593 $\pm$ 177	1393 $\pm$ 372	1455 $\pm$ 172	2222 $\pm$ 707
Striatum and habenula					
Nucleus accumbens	NAcc				
shell	shNAcc **	1068 $\pm$ 54	1240 $\pm$ 321	1560 $\pm$ 166	1474 $\pm$ 160
septal pole	spNAcc	1496 $\pm$ 193	949 $\pm$ 23	10608 $\pm$ 2223	13684 $\pm$ 4451
Caudate-putamen	CPu **				
dorsal	CPuD *	820 $\pm$ 35	791 $\pm$ 45	1095 $\pm$ 122	982 $\pm$ 32
ventral	CPuV *	1071 $\pm$ 60	866 $\pm$ 55	1630 $\pm$ 177	1310 $\pm$ 158
Habenula					
medial	mHab	935 $\pm$ 40	814 $\pm$ 35	1129 $\pm$ 134	1037 $\pm$ 118
lateral	lHab *	1042 $\pm$ 53	965 $\pm$ 57	1325 $\pm$ 91	1167 $\pm$ 72
Thalamus					
laterodorsal	ldThal **	5308 $\pm$ 1117	7582 $\pm$ 1027	504 $\pm$ 39	1224 $\pm$ 125
Olfactory and hindbrain					
Olfactory bulb					
external plexiform	OB *	1587 $\pm$ 48	1611 $\pm$ 48	3023 $\pm$ 224	3778 $\pm$ 944
Superior colliculus	SColl	862 $\pm$ 146	1227 $\pm$ 157	1323 $\pm$ 267	1218 $\pm$ 35
Periaqueductal gray	PAG	984 $\pm$ 93	821 $\pm$ 68	1529 $\pm$ 178	1197 $\pm$ 129
Dorsal raphe	DR **	10575 $\pm$ 547	10593 $\pm$ 1377	19083 $\pm$ 2604	16370 $\pm$ 1686

Region	Abbreviation	Meadow Female Mean $\pm$ SEM	Meadow Male Mean $\pm$ SEM	Prairie Female Mean $\pm$ SEM	Prairie Male Mean $\pm$ SEM
Locus coeruleus	LC	827 $\pm$ 96	895 $\pm$ 41	1363 $\pm$ 190	1041 $\pm$ 86
Pontine dorsal tegmental n.	PDTg	863 $\pm$ 51	758 $\pm$ 90	1417 $\pm$ 159	1035 $\pm$ 92
Principal motor nucleus of 5	Pr5	4317 $\pm$ 706	3721 $\pm$ 531	4692 $\pm$ 606	5250 $\pm$ 291
Cerebellum granular layer	CBg**	932 $\pm$ 77	742 $\pm$ 97	1315 $\pm$ 145	1301 $\pm$ 102

¹ Values represent mean DPM/mg tissue from n = 4 per group.

ND, not detected (<2 S.D. nonspecific binding);

* $P < 0.05$ species effect;

** $P < 0.01$ species effect;

[†] $P < 0.05$ sex effect.

Relative Comparison of CRFR₁ Binding across Six Species: Semiquantitative Analysis

Region	Meadow	Prairie	Montane	Pine	Rat ¹	Monkey ²
Cortex	+++	+++	++	++	+++	+++
Cerebellum	+++	+++	++	++	+++	+++
Olfactory bulb	+++++	++++	+++++	+++	+++	nr
Superior colliculus	+++++	++++	++++	+	++	nr
Lateral septum	++	++	++++	++++	0	0
Hippocampus	0	0	0	0	++	++++
Amygdala	+ / ++	+	+	0 / +	+++	++++
Periaqueductal gray	+	+	0	0	++++	nr
Habenula	++	++++	0	0	0	nr
Laterodorsal thalamus	0	0	++	0	++	nr
Locus coeruleus	+	+	+	0	0	+++
Dorsal raphe	0	0	++	++++	++	0
Nucleus accumbens, shell	+++	0 / +	++	0	++	nr

¹ Rat data are compiled from meta-analysis of CRFR₁ receptor binding reports from de Souza et al. (1985), de Souza et al. (1987), Primus et al. (1997), and Steckler and Holsboer (1999).

² Monkey data are summarized from Sanchez et al. (1999) and Grigoriadis et al. (1995).

For vole data, averaged across sex: 0, <1000 dpm/mg; +, 1000–2000 dpm/mg; ++, 2000–4000 dpm/mg; +++, 4000–6000 dpm/mg; +++++, 6000–10000 dpm/mg; ++++++, >10000 dpm/mg; nr, not reported.

TABLE 6

Relative Comparison of CRFR₂ Binding across Six Species: Semiquantitative Analysis

Region	Meadow	Prairie	Montane	Pine	Rat ¹	Monkey ²
Choroid plexus	++++	++++	++++	++++	++++	++++
Dorsal raphe	++++	++++	++++	++++	+++	++
Olfactory bulb	++	++	+	++	+++	nr
Cortex	++	++	+	+	+	+
Amygdala	++	++	+	+	+++	+++
Periaqueductal gray	++	++	0/+	+	+	nr
Locus coeruleus	++	++	0/+	+	0	0
Cerebellum	++	++	0/+	+	0	0
Lateral septum	++++	+++	++++	++++	++++	+
Hippocampus	++++	++	+	+	+	+++
Laterodorsal thalamus	0	0	++++	0/+	0	nr
Superior colliculus	++	++	+	+	0	nr
Nucleus accumbens, septal pole	++	+++	+	++++	0	nr

¹ Rat data are compiled from meta-analysis of CRFR₂ receptor binding reports from de Souza et al. (1987), Primus et al. (1997), Rominger et al. (1998), and Steckler and Holsboer (1999).

² Monkey data are summarized from Sanchez et al. (1999) and Grigoriadis et al. (1995).

For vole data, averaged across sex: 0, <1000 dpm/mg; +, 1000–2000 dpm/mg; ++, 2000–4000 dpm/mg; +++, 4000–6000 dpm/mg; +++++, 6000–10000 dpm/mg; ++++++, > 10000 dpm/mg; nr, not reported.