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Regional Differences in Mu and Kappa Opioid Receptor G-protein Activation in Brain in Male and Female Prairie Voles

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Abstract

Prairie voles are unusual mammals in that, like humans, they are capable of forming socially monogamous pair bonds, display biparental care, and engage in alloparental behaviors. Both mu and kappa opioid receptors are involved in behaviors that either establish and maintain, or result from pair bond formation in these animals. Mu and kappa opioid receptors both utilize inhibitory G-proteins as signal transduction mechanisms, however the efficacy by which these receptor subtypes stimulate G-protein signaling across the prairie vole neuraxis is not known. Utilizing [³⁵S]GTPγS autoradiography, we characterized the efficacy of G-protein stimulation in coronal sections throughout male and female prairie vole brain by DAMGO and U50,488H, selective mu and kappa opioid agonists, respectively. DAMGO stimulation was highest in forebrain, similar to that found with other rodent species. U-50,488H produced greater stimulation in prairie voles than is typically seen in mice and rats, particularly in select forebrain areas. DAMGO produced higher stimulation in the core versus the shell of the nucleus accumbens in females, while the distribution

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Thomas J. Martin wrote the manuscript and assisted with experimental design and data analysis.

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of U-50,488H stimulation was the opposite. There were no gender differences for U50,488H stimulation of G-protein activity across the regions examined, while DAMGO stimulation was greater in sections from females compared to those from males for nucleus accumbens core, entopeduncular nucleus, and hippocampus. These data suggest that the kappa opioid system may be more sensitive to manipulation in prairie voles compared to mice and rats, and that female prairie voles may be more sensitive to mu agonists in select brain regions than males.

Keywords

guanosine 5'-O[γ - ^{35} S] triphosphate; second messenger; signaling; autoradiography; DAMGO; U50; 488H; rodent; monogamy; social behavior; pair bonding

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Prairie voles (*Microtus orchogaster*) are relatively unusual among rodent species in that the male and female form lifelong, socially monogamous pair bonds after mating (Young et al., 2001, McGraw and Young, 2010, Johnson and Young, 2015). Prairie voles also, like humans, engage in alloparental behavior with both male and female involved in rearing of the young (Young et al., 2001, Young and Wang, 2004, Ahern and Young, 2009, Ahern et al., 2011). Due to these human-like social behaviors, the neurobiology of prairie voles related to formation of pair bonds has been studied in some detail and these studies have shown that oxytocin, vasopressin, and their receptors have prominent roles in pair bond formation and maintenance (Young et al., 2001, Lim et al., 2004, Young and Wang, 2004, Donaldson et al., 2010). Additionally, both mu and kappa opioid receptors are involved in these processes. Mu opioid receptor activation in limbic forebrain regions is required for pair bond formation in female prairie voles, while kappa opioid receptor activity is required for male-male aggression following pair bond formation with a female (Burkett et al., 2011, Burkett and Young, 2012, Resendez et al., 2012, Resendez et al., 2013). The role of opioid receptor subtypes in other brain areas in prairie vole behavior and neurobiology is an area of increased interest and investigation.

The neuroanatomical distribution of mu and kappa opioid receptor mRNA and protein has been examined in prairie vole brain, and their distribution is reasonably comparable to that found in the rat and mouse (Inoue et al., 2013). While opioid receptor density is generally highest in brain regions that are involved in the prominent pharmacological effects of opioids in the central nervous system, the efficacy by which opioids stimulate second messenger signaling through receptor activation does not always correlate with receptor density. The efficacy by which opioid agonists are able to activate second messenger systems is often determined using agonist stimulated binding of guanosine 5'-O[γ - ^{35} S] triphosphate ([^{35} S]GTP γ S) binding (Sim et al., 1995). This assay has the advantage in that it can be performed autoradiographically in tissue sections, and thereby agonist efficacy can be determined throughout the neuraxis for several compounds in the same animal. Such studies are valuable in that they determine relative agonist efficacies throughout the brain for a variety of receptor subtypes efficiently, and these experiments have been used extensively to compare receptor-G-protein coupling efficiency between animals or across pharmacological treatments (Breivogel et al., 1999, Sim-Selley et al., 2000).

In this study, we determined the efficacy of the mu opioid agonist DAMGO and the kappa opioid agonist U50,488H to stimulate G-protein activity using [^{35}S]GTP γ S binding in striatal membranes and using autoradiography in discrete regions throughout the brain in male and female prairie voles, and confirmed receptor selectivity for each agonist at appropriate concentrations of opioid antagonists. Additionally, anatomical differences between males and females were examined for both receptor subtypes.

2. Experimental Procedures

2.1. Subjects

A prairie vole colony was established at Wake Forest University Health Sciences (Winston-Salem, NC) using animals imported from a colony at Emory University (Atlanta, Georgia) originally derived from wild caught animals from Illinois. Subjects consisted of adult, sexually naïve male (N=8) and female (N=8) prairie voles (age 12–18 weeks) from the Wake Forest University Health Sciences colony weighing 40–60 g at time of sacrifice. All animals used for this study were housed in same sex groups of 2–4 animals and kept on a reversed 10:14 hr light:dark cycle (dark 03:00–17:00) in a temperature and humidity controlled vivarium within an AAALAC approved facility. The male and female pairs were housed separately but in the same room. Female prairie voles are induced into estrous in the presence of male urine, but without direct contact with males the ovaries are quiescent and these animals had not initiated an ovarian cycle. Males were sexually naïve as well. All animals were given *ad libitum* access to high fiber rabbit chow pellets (ProLab 5P25, LabDiet, St. Louis, MO), alfalfa cubes (Country Acres Feed, Brentwood, MO) and water. Bedding material consisted of ¼ inch corncob pellets (Bed-o’Cobs ¼”, The Andersons Lab Bedding, Maumee, OH), paper nesting material (Crink-I’Nest, The Andersons Lab Bedding) and cotton fiber nestlets (Ancare Corp., Bellmore, NY). Each cage also contained one red polycarbonate tube (3 inch diameter, 6 inch length, Bio-Serv, Flemington, NJ) for nesting and burrowing. All procedures were approved by the Animal Care and Use Committee of Wake Forest University (Winston-Salem, NC) and were in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Bethesda, MD).

2.2 Identification and verification of neuroanatomical brain regions

As there is currently no brain atlas available for the prairie vole, identification and verification of anatomical regions examined using the [^{35}S]GTP γ S autoradiography assay was accomplished using Nissl staining at 5 coronal levels of prairie vole brain in conjunction with a standard rat brain stereotaxic atlas (Paxinos and Watson, 1998). For this purpose four prairie voles were anesthetized with a lethal dose of sodium pentobarbital (100 mg/kg, i.p.). A blunt 23 gauge needle connected to a 20 ml syringe by 2 mm diameter silicon tubing was inserted into the left ventricle. The tip of the needle was stabilized with a hemostat and the vole was slowly perfused with 30 mL of 0.1 M phosphate buffered saline (PBS, pH = 7.4 at 4°C) followed by 30 mL of 4% formaldehyde fixative solution in 0.1M PBS. Brains were removed and post-fixed for 12 hours and then transferred to 30% sucrose solution in 0.1M PBS for cryoprotection. Serial 50 μm coronal brain sections were cut on a cryostat (Leica Microsystems, Buffalo Grove, IL) and thaw-mounted on plus slides for

histological processing. Nissl stained sections were rehydrated with 0.1M PBS for 10 min, post-fixed onto slides with 4% formaldehyde fixative for 10 min, rinsed in 0.1M PBS and ddH₂O, stained with 1% cresyl violet acetate solution (Sigma Aldrich, St. Lois, MO) for 15 min at room temperature, rinsed in distilled H₂O (1 min) and dehydrated in a series of 70, 95, and 100% alcohol solutions. Sections were then cleared in xylene and cover slips were affixed with DPX mounting media (Sigma-Aldrich). Bright field images were captured at 20× magnification with a DS Fi2 color camera on a Nikon Eclipse Ni microscopy system (Nikon Instruments Inc, Melville, NY) equipped with NIS-Elements basic research software with large image stitching capabilities.

2.3. Agonist-stimulated [³⁵S]GTPγS binding in vole striatal membranes

For [³⁵S]GTPγS binding in membranes, striata were dissected from 2 female vole brains on ice and frozen in aliquots at −80°C. Tissue samples were thawed, homogenized with a Tissumizer (Tekmar, Cincinnati OH) in cold TME buffer (50 mM Tris-HCl, 3 mM MgCl₂, 1 mM EGTA, pH 7.4) and centrifuged at 48,000 × g for 10 min at 4°C. Pellets were resuspended in membrane buffer and centrifuged again under identical conditions. After the second centrifugation, pellets were homogenized in TME assay buffer (50 mM Tris-HCl, 3 mM MgCl₂, 0.2 mM EGTA, 100 mM NaCl, pH 7.7). Concentration-effect curves of agonist-stimulated [³⁵S]GTPγS binding included 0.01–10 μM DAMGO (D-Ala₂,NMe-Phe₄,Gly-ol₅)-enkephalin, Tocris Bioscience, Ellisville, MO) or U-50,488H (*trans*-(−)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide HCl, Tocris Bioscience), 30 μM GDP, 0.05 nM [³⁵S]GTPγS (1200 Ci/mmol, Perkin Elmer, Waltham, MA), 100 nM DPCPX (8-cyclopentyl-1,3-dipropylxanthine, an adenosine A₁ receptor antagonist) (Moore et al., 2000), 5 μg membrane protein and TME assay buffer in a final volume of 1 ml. Basal binding was determined in the presence of GDP and absence of drug, and nonspecific binding was assessed in the presence of 10 μM GTPγS. Reactions were terminated by rapid filtration under vacuum through Whatman GF/B glass fiber filters followed by three washes with 3 ml cold 50 mM Tris-HCl buffer pH 7.7. Bound radioactivity was determined by liquid scintillation spectrophotometry at 95% efficiency for [³⁵S] after overnight extraction of the filters in 4 ml ScintiSafe Econo scintillation fluid. Data are reported as mean values ± SEM of three separate experiments, each performed in triplicate.

2.4. Agonist-stimulated [³⁵S]GTPγS autoradiography

Animals were euthanized by carbon dioxide asphyxiation followed by rapid decapitation. Brains were quickly removed and frozen in isopentane at −30 to −40°C and stored at −80°C until sectioned for autoradiography. Coronal sections (20 μm) were obtained using a cryostat at −22°C, and the sections were thawed onto glass slides for [³⁵S]GTPγS autoradiography (Sim et al., 1995). Brain sections were washed with TME buffer for 10 min at 25 °C prior to incubation with TME assay buffer containing 2 mM GDP for 10 min at 25°C. Sections were then incubated for two hours at 25°C in TME assay buffer containing 2 mM GDP, 100 nM DPCPX and 0.05 nM [³⁵S]GTPγS in the presence or absence of 3 μM of the mu agonist DAMGO or 1 μM of the kappa agonist U50,488H (Sim-Selley et al., 1999). Separate sections were incubated in a similar manner with the addition of 0.1 μM naloxone or 0.1 μM nor-BNI (nor-binaltorphimine, 17,17'-(dicyclopropylmethyl)-6,6',7,7'-6,6'-imino-7,7'-

binorphinan-3,4',14,14'-tetrol dihydrochloride, Tocris Bioscience) to inhibit mu- or kappa-opioid receptors, respectively. The sections were then washed twice with 50 mM Tris-HCl, pH 7.4 at 4 °C, rinsed once briefly with deionized water, and were exposed to phosphor-imaging screens overnight. Screen images were captured with a Cyclone Phosphor Imager (Perkin Elmer, Waltham, MA), and quantitative densitometric analysis was performed on regions of interest using NIH ImageJ software (National Institute of Health, Bethesda, MD, USA) in adjacent triplicate sections by an investigator that was blinded to the sex of each subject. Regions of interest were defined by user-defined settings in NIH Image software that selected areas of highest optical density. False color images were used for illustration purposes, not quantification; color scales were chosen based on the highest optical density obtained in all the brain sections, and were kept the same in all the remaining sections. Optical densities were quantitated by comparison with [^{14}C] brain paste standards and values corrected to nCi/g [^{35}S]. Data were expressed as net agonist-stimulated [^{35}S]GTP γ S binding. Statistical comparison of net binding between male and female brains was determined by ANOVA followed by planned comparisons within each brain region using a post-hoc t-test with Bonferroni correction, with statistical significance indicated by a p-value of 0.05/16 regions for DAMGO stimulation (corrected p-value of 0.0031) or 0.05/13 for U50,488H (corrected p-value of 0.0038). All chemicals were reagent grade and purchased from Fisher Scientific (Waltham, MA) or Sigma-Aldrich (St. Louis, MO) unless otherwise noted.

3. Results

To determine the neuroanatomical distribution of mu and kappa-activated G-proteins in the prairie vole brain, DAMGO and U50,488H were used as selective mu and kappa agonists, respectively, to stimulate [^{35}S]GTP γ S binding in brain sections. To determine the concentrations of agonists required to produce maximal stimulation of [^{35}S]GTP γ S, concentration-effect curves were generated in striatal membranes prepared from female vole brains. Results of [^{35}S]GTP γ S binding (Fig. 1) showed that DAMGO or U50,488H stimulated binding with EC_{50} values of 0.15 μM or 0.098 μM , respectively. To produce maximal stimulation, 3 μM DAMGO and 1 μM U50,488H were used in [^{35}S]GTP γ S autoradiography experiments.

To confirm the pharmacological specificity of these agonists in prairie vole, [^{35}S]GTP γ S autoradiography was performed in the presence of selective mu and kappa antagonists. Fig. 2 shows representative autoradiograms from the level of forebrain and cerebellum; this experiment was repeated in the other coronal levels used for examining [^{35}S]GTP γ S stimulated binding with similar results (not shown). In these sections, the mu agonist DAMGO (3 μM) produced high stimulation of [^{35}S]GTP γ S binding over basal (identical conditions with no agonist added to the incubation buffer) in nucleus accumbens and caudate-putamen, with moderate stimulation in cingulate cortex and in cerebellum. All of this specific DAMGO-stimulated binding was blocked by addition of naloxone at a concentration (0.1 μM) that is relatively mu selective, while addition of the kappa antagonist nor-BNI (0.1 μM) had no effect on DAMGO-stimulated binding (Fig. 2, top row). Similarly, stimulation of [^{35}S]GTP γ S binding by the kappa agonist U50,488H (1 μM) in nucleus accumbens, caudate and cerebellum was completely blocked by nor-BNI (0.1 μM), while

naloxone (0.1 μ M) had no effect (Fig. 2, bottom row). These results confirmed that DAMGO and U50,488H were selective in activating mu- and kappa-opioid receptor-coupled G-proteins in prairie vole brain.

Detailed quantitative analysis was performed on [^{35} S]GTP γ S autoradiograms of prairie vole brains at five different coronal levels, comparing the distribution of net mu- and kappa-opioid receptor stimulated [^{35} S]GTP γ S binding. Representative autoradiograms are shown in Fig. 3; results from quantitative densitometric analysis of autoradiograms are shown in Table 1. Representative Nissl-stained sections from prairie vole brain (Fig. 4) provide a guide to the principal brain structures identified by [^{35} S]GTP γ S autoradiography. The highest levels of both mu- and kappa-opioid receptor stimulated [^{35} S]GTP γ S binding occurred in forebrain (Fig. 3, top row). For mu, high levels of activity (>150 nCi/g net stimulation) were observed in nucleus accumbens, caudate-putamen, and cingulate cortex, and no activity in claustrum. In nucleus accumbens, higher levels of mu activity were observed in core compared to shell in females. For kappa, high levels of activity were observed in nucleus accumbens shell and (in contrast to mu activity) claustrum, with moderate (between 150 and 95 nCi/g net stimulation) kappa activity in caudate-putamen and nucleus accumbens core and (unlike mu) no kappa activity in cingulate cortex. In contrast to mu activity, kappa activity in nucleus accumbens was higher in shell compared to core.

At the middle rostrocaudal level of the striatum (Fig. 3, second row), once again the highest levels of both mu- and kappa-stimulated [^{35} S]GTP γ S binding were observed in caudate-putamen. For mu, moderate activity was observed in lateral septum and ventral pallidum. For kappa, in contrast to mu, a high level of activity was observed in ventral pallidum, but none in lateral septum.

At the level of the anterior diencephalon (Fig. 3, third row), mu activity was moderate in a wide variety of structures, including amygdala, medial thalamus, and ventromedial hypothalamus. Low levels of kappa activity (>95 nCi/g) were observed in ventromedial hypothalamus with moderate activity observed in a thin dorsomedial band of the hypothalamus. Such a band was not distinguishable for mu activity apart from the other aspects of the hypothalamus. Unlike mu opioid receptor activation, low kappa-opioid receptor activation was seen in the amygdala, medial thalamus, and ventromedial hypothalamus.

At the level of the posterior diencephalon (Fig. 3, fourth row), moderate levels of mu-opioid receptor stimulated [^{35} S]GTP γ S binding were observed in central gray, hippocampus, and interpeduncular nucleus. Low levels of mu activity were observed in medial geniculate and superior colliculus. Low levels of kappa-opioid receptor activity were observed in central gray and hippocampus, however unlike mu no kappa activity was observed in medial geniculate, interpeduncular nucleus or superior colliculus.

In the hindbrain (Fig. 3, last row), low but measurable levels of mu-opioid receptor stimulated [^{35} S]GTP γ S binding were observed in both cerebellum and parabrachial nucleus. Low levels of kappa activity were observed in both of these regions as well, with the level of net kappa activity in both regions barely above the minimal level of accurate quantification.

The finding of significant DAMGO-stimulated activity in cerebellum is quite different from rat and mouse cerebellum, where mu receptor activity is notably absent (Sim et al, 1995), but agrees with the finding of significant mu receptor radioligand binding reported previously in prairie vole cerebellum (Inoue et al., 2013). The finding (Fig. 2) that the DAMGO-stimulated [^{35} S]GTP γ S is blocked specifically by naloxone confirms that this DAMGO stimulation in cerebellum is mediated by mu receptors.

Quantitative densitometric data was also used to determine whether there were any significant differences between male and female for either mu- or kappa-opioid receptor stimulated [^{35} S]GTP γ S binding in the regions examined. Table 1 shows a comparison of mu and kappa activities in different brain regions from male and female voles. For mu-stimulated [^{35} S]GTP γ S binding, significant differences between male and female vole brains were observed in only three regions: nucleus accumbens core ($p < 0.0001$ male vs. female), interpeduncular nucleus ($p < 0.0001$) and hippocampus ($p < 0.002$). In all three regions, the levels of net mu-stimulated [^{35} S]GTP γ S binding were significantly higher in females compared to males. In contrast, there were no significant differences between males and females for kappa-opioid receptor stimulated [^{35} S]GTP γ S binding in any region tested.

4. Discussion

This initial description of the neuroanatomical distribution of mu and kappa opioid receptor G-protein activation by selective agonists suggests that interesting differences exist between prairie voles and other rodents that typically serve as subjects for preclinical behavioral and physiological research. One notable difference is the relatively robust activation of G-proteins by a kappa agonist, and the regions identified as being relatively more sensitive to U50,488H are similar to those identified as having measurable amounts of kappa opioid receptors using receptor autoradiography (Resendez et al., 2012). Kappa opioid receptors are sparse and kappa agonists typically produce quite modest activation of G-protein signaling as measured by [^{35} S]GTP γ S binding in both rats and mice (Hyytia et al., 1999, Slowe et al., 1999, Park et al., 2000, Piras et al., 2010). In the prairie vole, the ability of U50,488H to stimulate [^{35} S]GTP γ S binding was particularly robust in the accumbens shell relative to DAMGO when compared to the above cited studies in rats and mice (Table 1). In both male and female prairie voles, injection of nor-BNI into the shell of the accumbens inhibited selective aggression using a resident-intruder paradigm, suggesting a key role for kappa opioid receptors in maintaining pair bonds for both sexes (Resendez et al., 2012). Interestingly, these investigators reported that injection of nor-BNI into the accumbens core enhanced selective aggression only in females (Resendez et al., 2012) while in the present study we find no differences in activation of G-proteins by a kappa agonist between male and females, suggesting that these gender differences in the core are likely not explained by fundamental differences in the efficiency of kappa opioid receptor-G protein coupling. This may indicate that kappa opioid receptors are more intimately involved in reinforcement systems in the prairie vole relatively to other typical laboratory rodent species, as has been suggested by the involvement of kappa opioid receptors in male-male aggression following pair bond formation (Resendez et al., 2012). The present data also suggest that the ventral pallidum may be a region that merits exploration for involvement of kappa-opioid receptors in prairie vole neurobiology (Table 1). The ventral pallidum is thought to coordinate motor

responses to cognitive processes, and in the rat this region receives coordinated input from the nucleus accumbens, amygdala, and ventral tegmental area. Stimulatory amygdala input to the ventral pallidum is negatively modulated by dopaminergic input from the ventral tegmental area in the rat (Maslowski-Cobuzzi and Napier, 1994). Kappa opioid agonists decrease dopaminergic release from ventral tegmental neurons in rats via a presynaptic action, and thereby likely serve to mitigate the influence of ventral tegmental input into this region (Mitrovic and Napier, 2002). Kappa opioid receptors may therefore serve as a regulator of the coordination between amygdala and ventral tegmental area influence over motor responses and behavioral activity in the prairie vole, and the present data suggest that the influence of kappa opioid receptors in this region may be greater than those of mu-opioid receptors. Previous studies have shown that kappa opioid receptors in the ventral pallidum, unlike in the accumbens, do not appear to mediate male-male aggression following pair bond formation however (Resendez et al., 2012).

The efficacy by which DAMGO stimulated [³⁵S]GTPγS binding and the relative distribution throughout the prairie vole brain was similar to that reported previously for rats and mice (Sim et al., 1995, Hyytia et al., 1999, Park et al., 2000). The distribution of DAMGO-stimulated [³⁵S]GTPγS binding in male prairie vole brain reported here is likewise similar to the distribution of mu-opioid receptor binding reported previously using receptor autoradiography (Resendez et al., 2012, Inoue et al., 2013). Mu-opioid binding has also been analyzed in female forebrain and the distribution pattern is similar to that reported here for DAMGO-stimulated [³⁵S]GTPγS binding, particularly comparing nucleus accumbens core with ventral accumbens shell (Resendez et al., 2013). Unlike kappa opioid receptor stimulation however, there were three brain regions that showed distinct differences between male and female prairie voles for DAMGO-stimulated [³⁵S]GTPγS binding (Table 1). These regions were the nucleus accumbens core, interpeduncular nucleus, and hippocampus, with females showing a greater net stimulation of [³⁵S]GTPγS binding with DAMGO than males. The involvement of mu-opioid receptors in the unique behavioral phenotype of pair bond formation has been explored in the prairie vole. In females, antagonism of mu-opioid receptors in the caudate, but not in the nucleus accumbens shell, prevents pair bond formation (Burkett et al., 2011). When comparisons were made between the effects of mu-opioid antagonists on mating behavior and pair bond formation, it was shown that mu-opioid receptors within the caudate mediate both mating and pair bonding, while antagonism of those in the dorsomedial accumbens shell disrupt pair bond formation without affecting mating behavior (Resendez et al., 2013). There are some discrepancies between these two studies, however it is clear that mu opioid receptors in the dorsal striatum and dorsal accumbens are involved in pair bond formation in female prairie voles, but not those in the ventral accumbens shell. Similar studies have not been reported in male prairie voles, however the present data would predict that in males the anatomical selectivity may be relatively less pronounced than in females if mu opioid receptor G-protein activation is predictive of *in vivo* pharmacology. Studies have not been reported examining the function of mu opioid receptors in behaviors specifically related to the interpeduncular nucleus or hippocampus in prairie voles, however the present data likewise predict that females could potentially be more sensitive to manipulation of mu opioid activity in these regions than males. The interpeduncular nucleus is part of the basal ganglia and is thought to mediate the

effects of opioids on limbic function to some extent, while the hippocampus is typically associated with learning and memory. Differences in mu opioid receptor G-protein coupling between males and females in these regions could likewise suggest increased sensitivity to manipulation of these behaviors by endogenous or exogenous mu agonists in female prairie voles relative to males.

In the present study, the ability of mu and kappa opioid agonists to stimulate G-protein activation was examined only in the monogamous prairie vole. There are a limited number of studies demonstrating differential effects of opioids and opioid antagonists in modulating social behaviors in prairie voles relative to non-monogamous strains such as montane voles or meadow voles. However studies that directly compare opioid receptor densities and localization between prairie voles and these non-monogamous vole strains have not been documented extensively (but see Inoue et al., 2013). The present study suggests that [³⁵S]GTPγS autoradiography might be useful in determining if social behaviors specific to monogamous versus non-monogamous voles correlate with the efficiency of mu and/or kappa opioid receptor coupling to G-proteins within discrete brain regions.

Conclusion

The distribution of mu- and kappa-opioid receptor G-protein activation in the prairie vole is similar to that reported previously for each receptor subtype using receptor autoradiography. Gender differences for stimulation of [³⁵S]GTPγS binding were confined to mu-opioid receptors, with females displaying greater activation of males in only 3 discrete brain regions which may have important implications for gender differences in behavior that should be explored in futures studies. Further, we validate here the autoradiographic method for opioid receptor subtype stimulation of [³⁵S]GTPγS binding in prairie vole brain that can be used in future studies to determine changes in opioid receptor signaling relevant to social behaviors as well as opioid addiction and pain management.

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Abbreviations

DAMGO	[D-Ala ₂ ,NMe-Phe ₄ ,Gly-ol ₅]-enkephalin
[³⁵S]GTPγS	guanosine 5'-O[γ- ³⁵ S] triphosphate
NAc	nucleus accumbens
nor-BNI	nor-binaltorphimine

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Highlights

- Opioid stimulation of [^{35} S]GTP γ S binding was validated in prairie vole brain.
- Mu and kappa opioid stimulation was consistent with reported receptor localization.
- Females displayed higher mu stimulated [^{35} S]GTP γ S binding than males in 3 regions.
- No gender differences were found for kappa stimulated [^{35} S]GTP γ S binding.

Fig. 1. Concentration-effect curves of DAMGO and U50,488H in stimulating [^{35} S]GTP γ S binding in striatal membranes from female prairie vole brains

Membranes were prepared and assayed for agonist-stimulated [^{35} S]GTP γ S binding as described in Methods, using 0.01–10 μ M concentrations of DAMGO and U50,488H. Results are expressed as per cent stimulation over basal binding, and represent mean values $\pm$ SEM of three different assays each performed in triplicate.

Fig. 2. Pharmacological specificity of DAMGO- and U50,488H-stimulated [^{35} S]GTP γ S binding in vole brain

Sections of male prairie vole forebrain were incubated as described in Experimental Procedures with 0.05 nM [^{35} S]GTP γ S and 2 mM GDP with DAMGO (3 μ M) as a mu agonist or U50,488H (1 μ M) as a kappa agonist, with and without the mu antagonist naloxone (0.1 μ M) or the kappa antagonist nor-BNI (0.1 μ M). Similar results were obtained at 3 additional coronal levels (not shown).

Fig. 3. Representative autoradiograms of mu- and kappa-stimulated $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding
Coronal sections are shown of female prairie vole brains at five brain levels (top to bottom); forebrain, mid-striatum, anterior diencephalon, posterior diencephalon, and hindbrain. Sections were incubated as described in Experimental Procedures with 0.05 nM $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ and 2 mM GDP with 3 μM DAMGO or 1 μM U50,488H.

Fig. 4. Nissl stained sections of prairie vole brain

Sections from female prairie vole brain are shown with identifying regional labels at 5 coronal levels.

Table 1

Quantitative densitometric analysis of mu- and kappa-opioid-stimulated [^{35}S]GTP γ S binding in male and female prairie vole brain sections by autoradiography.

Region	Net agonist-stimulated [^{35}S]GTP γ S binding, nCi/g:			
	Female		Male	
	Mu	Kappa	Mu	Kappa
Cingulate cortex	168 $\pm$ 8.1		178 $\pm$ 6.4	
Clastrum		168 $\pm$ 4.6		178 $\pm$ 6.9
Nucleus accumbens core	281 $\pm$ 8.1	123 $\pm$ 5.2	240 $\pm$ 5.8*	137 $\pm$ 6.4
Nucleus accumbens shell	219 $\pm$ 8.1	168 $\pm$ 5.2	237 $\pm$ 9.2	176 $\pm$ 6.9
Caudate-putamen	154 $\pm$ 5.2	99.9 $\pm$ 3.6	152 $\pm$ 4.6	106 $\pm$ 3.5
Lateral septum	108 $\pm$ 5.8		116 $\pm$ 5.8	
Ventral pallidum	97 $\pm$ 2.9	167 $\pm$ 7.5	96.2 $\pm$ 2.5	176 $\pm$ 8.7
Medial thalamus	145 $\pm$ 4.0	76.1 $\pm$ 3.1	126 $\pm$ 2.3	80.6 $\pm$ 5.5
Dorsomedial hypothalamus		106 $\pm$ 6.4		116 $\pm$ 4.6
Ventromedial hypothalamus	111 $\pm$ 3.5	61.3 $\pm$ 2.9	101 $\pm$ 3.5	74.8 $\pm$ 5.0
Amygdala	106 $\pm$ 4.6	61.3 $\pm$ 3.8	118 $\pm$ 5.8	59.6 $\pm$ 4.6
Central gray	138 $\pm$ 2.9	78.1 $\pm$ 2.5	135 $\pm$ 2.3	80.7 $\pm$ 3.3
Superior colliculus	86.7 $\pm$ 2.6		84.9 $\pm$ 3.4	
Medial geniculate	81.3 $\pm$ 3.4		79.1 $\pm$ 3.2	
Hippocampus	143 $\pm$ 4.6	91.8 $\pm$ 4.2	115 $\pm$ 5.8**	76.1 $\pm$ 5.1
Interpeduncular nucleus	149 $\pm$ 6.9		113 $\pm$ 5.8*	
Cerebellum	84.7 $\pm$ 3.4	27.2 $\pm$ 1.9	89.6 $\pm$ 2.9	31.8 $\pm$ 3.9
Parabrachial nucleus	82.8 $\pm$ 4.2	39.8 $\pm$ 3.2	91.7 $\pm$ 2.0	50.3 $\pm$ 3.1

Coronal sections from prairie vole brains (N=8, females; N=8, males) were incubated with 3 μM DAMGO (mu) or 1 μM U50,488H (kappa) with [^{35}S]GTP γ S as described in Methods. After scanning autoradiograms on phosphor screens, quantitative densitometry was performed using NIH Image with ^{14}C standards (corrected for ^{35}S) to generate data expressed as nCi/g tissue. Regions with blank data contained levels of net agonist-stimulated binding that were too low (<20 nCi/g) to be accurately determined. Data are mean values $\pm$ SEM from triplicate sections from each brain;

*, $p < 0.0001$ male vs. female;

**, $p = 0.0014$ male vs. female.