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NEUROANATOMICAL DISTRIBUTION OF μ-OPIOID RECEPTOR mRNA AND BINDING IN MONOGAMOUS PRAIRIE VOLES (*MICROTUS OCHROGASTER*) AND NON-MONOGAMOUS MEADOW VOLES (*MICROTUS PENNSYLVANICUS*)

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Abstract—The opiate system has long been implicated in the rewarding properties of social interactions. In particular, the μ-opioid receptor (MOR) mediates multiple forms of social attachment, including the attachment of offspring to the mother and social bonding between mates. We have previously shown that MOR in the caudate-putamen is involved in partner preference formation in monogamous prairie voles. Here, using *in situ* hybridization and receptor autoradiography, we mapped in detail the distribution of MOR mRNA and ligand binding in monogamous prairie vole brains and compared MOR binding density with that of promiscuous meadow vole brains. Comparison of MOR binding in these closely related species with distinctly different social behavior revealed that while the distribution of MOR is similar, prairie voles have significantly higher densities of MOR than meadow voles in a majority of regions in the forebrain, including the caudate-putamen, nucleus accumbens shell, lateral septum and several thalamic nuclei, including the anteroventral and anteromedial thalamic nuclei. These differences in MOR expression between prairie and meadow voles could potentially contribute to species differences in behavior, including social attachment.

Key words: pair bonding, social behavior, social attachment.

INTRODUCTION

The brain opiate system modulates a number of fundamental processes including pain, analgesia, and the rewarding properties of food, water, sex, and addictive drugs (Turkish and Cooper, 1983; Agmo and Berenfeld, 1990; Yeomans and Gray, 1996; Sora et al., 1997; Gerrits et al., 2003; Fields, 2007). In addition to the effects on analgesia and reward, the opiate system has been proposed to play an important role in modulating social reward, including maternal behavior, social motivation, and social attachments (Nelson and Panksepp, 1998). Social attachment has many parallels with opiate addiction (Panksepp et al., 1978; Insel, 2003; Burkett and Young, 2012). For instance, the distress evoked by separation of the offspring from the parent shares psychological symptoms with opiate withdrawal, can be induced with opioid antagonists, and can be alleviated with opioid agonists (Herman and Panksepp, 1978; Panksepp et al., 1978, 1980; Warnick et al., 2005). Furthermore, in Rhesus macaques, acute administration of an opiate antagonist increases maternal and affiliative behavior, while morphine decreases these behaviors (Fabre-Nys et al., 1982; Kalin et al., 1988; Martel et al., 1993).

The opiate system is activated either by exogenous opiate drugs such as morphine and heroin, or by endogenous neuropeptides such as endorphin, enkephalin, and dynorphin (Le Merrer et al., 2009). The targets of these neuropeptides are the opioid receptors, mu (μ), kappa (κ), and delta (δ). The μ-opioid receptor (MOR) seems to be principally involved in modulating the hedonic properties of many addictive drugs as well as endogenous reward and pain (van Ree et al., 1999; Leknes and Tracey, 2008; Loyd et al., 2008). MOR is also the principal receptor implicated in social reward. MOR knockout mice show decreased social exploration toward opposite-sex conspecifics, decreased response to social defeat, and maternal attachment deficits (Moles et al., 2004; Komatsu et al., 2011; Wohr et al., 2011). Rhesus macaques with the C77G polymorphism of the MOR gene show increased infant–mother attachment and increased maternal care (Barr et al., 2008; Higham et al., 2011). This role for MOR seems to be conserved in humans, where an analogous polymorphism in the MOR gene has been correlated with increased social affection, increased responses to social rejection and social rewards, and altered social attachment (Barr et al., 2008; Way et al., 2009; Higham et al., 2011; Troisi et al., 2012).

Recent studies in voles have provided great insights into the neurobiological mechanisms underlying social bonding between mates, or pair bonding (Young and Wang, 2004; McGraw and Young, 2010). Closely related species of voles show strikingly different social

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Abbreviations: AChE, acetylcholinesterase; CP, caudate-putamen; CRF, corticotropin-releasing factor; CTAP, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2; KOR, κ-opioid receptor; LC, locus coeruleus; LS, lateral septal nucleus; MnR, median raphe; MOR, μ-opioid receptor; NAcsh, nucleus accumbens shell; NTX, naltrexone; OTR, oxytocin receptor; PrL, prelimbic cortex; SNR, substantia nigra pars reticulata.

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attachment behaviors (Thomas and Birney, 1979; Madison, 1980; Getz et al., 1981). Socially monogamous prairie voles (*Microtus ochrogaster*) are highly affiliative, display biparental care, and form selective pair bonds between mating partners. By contrast, meadow voles (*Microtus pennsylvanicus*) are asocial and mate promiscuously without forming pair bonds. Pharmacological and genetic manipulation studies have revealed that oxytocin, vasopressin, dopamine and corticotropin-releasing factor (CRF) act in the mesolimbic reward and reinforcement system to facilitate the formation of the social bond between mates (Insel and Young, 2001; Young et al., 2001; Liu and Wang, 2003; Lim et al., 2004, 2007; Ross et al., 2009). Species differences in the expression levels, distribution or regulation of receptors for these neuromodulators have been implicated in the differences in social behavior between prairie and meadow voles (Lim et al., 2004, 2005b, 2007; Ross et al., 2009). More recently, we have shown that MOR in the dorsal caudate-putamen (CP) also plays a critical role in pair bond formation (Burkett et al., 2011). Infusion of D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2 (CTAP), a selective MOR antagonist, into the dorsal CP of female prairie voles just prior to pairing with a male prevents the development of a partner preference, a laboratory proxy for the pair bond (Burkett et al., 2011). Furthermore, peripheral infusion of naltrexone, a nonselective opioid antagonist, results in a partner aversion. While the partner aversion observed after peripheral infusion of opioid antagonist is likely the consequence of the aversive effects of opioid antagonists, the lack of a partner aversion following CP infusion of MOR antagonist suggests that MOR plays a specific role in mating-induced partner preference formation, rather than having non-specific aversive effects.

Recent studies have provided some information on MOR binding, but not mRNA distribution, in prairie vole (Burkett et al., 2011; Resendez et al., 2012). To better understand the MOR system in the prairie vole, we here characterize the distribution of MOR binding and mRNA throughout the prairie vole brain using receptor autoradiography and *in situ* hybridization. In addition, to explore potential species differences in MOR expression that may be related to species differences in social attachment behaviors, we compare MOR binding density between two vole species with different patterns of social attachment, prairie and meadow voles.

EXPERIMENTAL PROCEDURES

Animals

Adult prairie and meadow voles from 10 weeks to 9 months of age were obtained from our breeding colonies at the Yerkes National Primate Research Center. All prairie voles were descended from a wild caught population in Illinois, USA, and meadow voles were descended from a wild caught population from southeastern USA. All cages were maintained on a 14:10-light:dark cycle with the temperature at 20 °C. After weaning at 21 days of age, subjects were housed in same sex sibling pairs or trios with water and Purina rabbit chow provided *ad libitum*. All subjects in this study were sexually naïve.

All experiments were done in accordance with the Institutional Animal Care and Use Committee at Emory University.

MOR autoradiography

Brains were dissected from male prairie and meadow voles ($N = 6$ each). Brain slices (20 μm) were prepared for MOR autoradiography using 1 nM [Tyr-3,5-^{3}H(N)]-DAMGO ([^{3}H]DAMGO, Perkin–Elmer, MA) and analyzed as described previously (Loyd et al., 2008). Noncompetitive MOR binding to brain slices was measured using [^{3}H]DAMGO alone. As a control, competitive binding was measured on adjacent sections using [^{3}H]DAMGO in the presence of either a μ-opioid selective antagonist, CTAP (10 μM) or a nonselective opioid antagonist, naltrexone (NTX, 10 μM), to demonstrate that the ligands bound to the vole MOR as described previously in rat. After sixty days exposure to phosphor imaging plates, signals were acquired using a BAS 5000 phosphor imaging scanner (Fujifilm, Tokyo, Japan), and quantified using Fujifilm MultiGauge software. Signal intensity for each region was calculated by averaging the quantum level/pixel^2 from two or three sections bilaterally per animal. Averaged signals in the corpus callosum were used as background and subtracted from each mean value to yield specific binding. Digital images were cropped, transferred to Adobe Photoshop CS (Adobe Systems, San Jose, CA) and brightness and contrast were equally adjusted for all the images from both the prairie and meadow vole brains.

Acetylcholinesterase (AChE) stain

Following MOR autoradiography, slices were counterstained for acetylcholinesterase for accurate identification of the brain regions as described previously (Lim et al., 2005a).

MOR *in situ* hybridization

Sense and antisense ^{35}S-UTP-labeled RNA probes for MOR mRNA were generated as described previously (Inoue et al., 2004; Burkett et al., 2011). The RNA probe was complementary to the prairie vole MOR sequence corresponding to base pairs 341–1409 of mouse MOR cDNA (Genbank accession number U19380). Twenty-μm cryosections adjacent to the slices used for MOR autoradiography were hybridized with the probes, and then were exposed to Kodak BioMax MR films for 5 weeks. The slides were then coated with Kodak NTB emulsion. After 4 months of exposure, sections were developed in Kodak D-19 and fixed with Kodak rapid fixer. Sections were then counterstained with Thionin. Regional MOR mRNA expressions were graded as very high (++++), high (+++), intermediate (++) and low (+) to give semi-quantitative estimates of signal strength. For film autoradiograms, digital images were obtained using a light box and a SPOT camera (Diagnostic Instruments, Sterling Heights, MI) connected to a computer. Bright-field and dark-field microscope images were taken with Nikon E800 microscope and SPOT camera setup. Brightness and