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Comparative distribution of central neuropeptide Y (NPY) in the prairie (*Microtus ochrogaster*) and meadow (*M. pennsylvanicus*) vole☆

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ABSTRACT

Neuropeptide Y (NPY) has been implicated as a modulator of social behavior, often in a species-specific manner. Comparative studies of closely related vole species are particularly useful for identifying neural systems involved in social behaviors in both voles and humans. In the present study, immunohistochemistry was performed to compare NPY-like immunoreactivity (-ir) in brain tissue of the socially monogamous prairie vole and non-monogamous meadow vole. Species differences in NPY-ir were observed in a number of regions including the cortex, extended amygdala, septal area, suprachiasmatic nucleus, and intergeniculate leaf. Meadow voles had higher NPY-ir in all these regions as compared to prairie voles. No differences were observed in the striatum or hippocampus. The extended amygdala and lateral septum are regions that play a key role in regulation of monogamous behaviors such as pair bonding and paternal care. The present study suggests NPY in these regions may be an additional modulator of these species-specific social behaviors. Meadow voles had moderately higher NPY-ir in a number of hypothalamic regions, especially in the suprachiasmatic nucleus. Meadow voles also had much higher levels of NPY-ir in the intergeniculate leaflet, another key region in the regulation of circadian rhythms. Overall, species differences in NPY-ir were observed in a number of brain regions implicated in emotion, stress, circadian, and social behaviors. These findings provide additional support for a role for the NPY system in species-typical social behaviors.

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1. Introduction

Social behaviors such as selective emotional attachment ("pair bonding") and biparental care play a critical role in human behavior and society, but are rare among mammals. Only 3% of mammalian species display these and other behaviors associated with monogamy [35]. Monogamous animal models are a useful tool for understanding the neurobiology of social behaviors not present in more common laboratory animals such as rats and mice [42]. The prairie vole (*Microtus ochrogaster*) is an arvicoline rodent that displays a suite of behaviors associated with social monogamy, including pair bonding, biparental care, and mate guarding [9,48]. In contrast, the closely related meadow vole species (*Microtus pennsylvanicus*), displays typical mammalian behavior associated with a promiscuous breeding system [48,58]. Despite these striking differences in social organization, these species are similar in morphology and in non-social behaviors [54,58].

Comparative studies between these species are particularly useful for identifying neural systems involved in social behaviors associated with monogamy. For example, *Microtus* species show species differences that reflect social organization in a number of peptide systems including arginine vasopressin (V1a receptor: [33,37]), oxytocin (OT receptor: [32,37]), corticotropin releasing factor (CRF, urocortin 1, and receptor subtypes 1 and 2: [38,39]) and CART (cocaine and amphetamine regulated transcript) peptide [28]. This approach has been largely successful in identifying neural

Abbreviations: AHA, anterior hypothalamic area; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CART, cocaine and amphetamine regulated transcript; CeA, central amygdala; DG, dentate gyrus; DMH, dorsomedial hypothalamus; GP, globus pallidus; IGL, intergeniculate leaflet; IPAC, interstitial nucleus of the posterior limb of the anterior commissure; LGN, lateral geniculate nucleus; LPOA, lateral preoptic area; LS, lateral septum (d, dorsal, i, intermediate, v, ventral); MeA, medial amygdala; MPN, medial preoptic nucleus; MPOA, medial preoptic area; MS, medial septum; NAc, nucleus accumbens; NPF, neuropeptide F; NPY, neuropeptide Y; NPY-ir, NPY-like immunoreactivity; PeV, periventricular nucleus of the hypothalamus; PVN, paraventricular nucleus of the hypothalamus; PV thal, paraventricular thalamic nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; VMH, ventromedial hypothalamus; VP, ventral pallidum.

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systems involved in modulating social behavior in prairie voles [26,31,50,53,65] with consistent findings in humans [17,25,62], and has demonstrated high translational and clinical potential [30,44]

Neuroepeptide Y (NPY) is involved in a wide array of behavioral processes including memory [60], stress and anxiety [60,67], circadian rhythms [2], hypertension [5], feeding [13,43], and alcohol drinking [12,59]. There are also a number of studies in a diversity of species implicating a role of this peptide in social behavior. Among invertebrates, the NPY system is implicated in social feeding behaviors. Wild strains of *Caenorhabditis elegans* display either social or solitary feeding behavior. This naturally occurring variation in feeding strategy is directly associated with two isoforms of the NPY receptor homolog gene *npr-1* [15]. Experimental manipulation of this gene can entirely shift the feeding behavior of the organism to an opposite strategy. Thus, a single gene in the NPY system can induce or prevent expression of social feeding behavior in this species. Social feeding strategies are also regulated developmentally by the NPY homolog neuropeptide F (NPF) in *Drosophila* [66]. The NPF system of the fruit fly is also highly responsive to the social environment, as mating increases central levels of each NPF and NPF mRNA, whereas sexual deprivation or rejection decreases these measures [55]. Research on social behavior and NPY in mammals has been primarily limited to the social interaction test in rats and mice. In this test, social investigation of a novel conspecific is measured. The traditional interpretation is that increased exploration is indicative of decreased anxiety. There is robust evidence for a role of NPY and its receptors in the social interaction test, with NPY promoting social interaction or preventing stress-induced decreases in interaction [20,45,52]. NPY has also been shown to decrease sexual behavior in male rats [13]. These studies across a variety of taxa and behaviors support a modulatory role for NPY in social behavior, often in a species-specific manner.

Given the likely role of NPY in social behavior, we aimed to compare the distribution of NPY in two closely related species that differ in social systems: the meadow vole (*M. pennsylvanicus*) and prairie vole (*M. ochrogaster*). In the present study, we analyzed brain tissue of meadow and prairie voles to perform a semi-quantitative comparison of NPY-like immunoreactivity (NPY-ir) between these species.

2. Experimental procedure

2.1. Animals

Voles used in these experiments were bred in the colony at Emory University, and housed in same-sex duos or trios until adulthood. At 100–120 days of age, 3 experimentally naïve voles of each sex and species (12 total) were euthanized by CO_2 inhalation followed by transcardial perfusion with 4% paraformaldehyde in phosphate-buffered saline (PBS). Brains were removed and post-fixed in the same solution, and then preserved in 30% sucrose in PBS. The brains were shipped on ice to Oregon Health & Science University where the immunohistochemistry and analyses were performed. The protocol was approved by the Emory Institutional Animal Care and Use Committee (protocol number 205-2007Y).

2.2. Immunohistochemistry

Tissue was sliced into 40-micron sections and stored in 0.1% sodium azide (NaN_3) in PBS until time of assay. Floating sections were rinsed in PBS and then incubated in 0.3% hydrogen peroxide for 15 min. Sections were then rinsed again in PBS and incubated in a block solution of 2% bovine serum albumin (BSA) and 5 mg/mL heparin in a 0.003% Triton X100 and PBS solution for 5 h. After blocking, sections were incubated overnight with a 1:50,000 dilution of rabbit polyclonal primary antibody (Anti-Neuropeptide Y, N9528, Sigma Aldrich, St. Louis, Missouri) in PBS with Triton and BSA. A parallel reaction to investigate non-specific binding was performed without the primary antibody, and no immunoreactivity was observed in the regions of interest described herein. Following overnight incubation, tissue was washed in PBS and incubated with a biotinylated-goat anti-rabbit secondary antibody in PBS and Triton for 1 h. Tissue was then washed in PBS and incubated for 1 h in A/B solution, then washed again in PBS. Tissue was stained using a DAB reaction and mounted the day of assay. All immunohistochemistry was performed at room temperature, and all tissue was assayed at the same time. All slides were coded by a party not performing analysis, and the code was not broken until data collection was complete.

For each region, we aimed to analyze photographs from both hemispheres of two serial sections, for an average of four sections per subject. In only a few cases, less than four sections were analyzed due to tissue quality or availability. Regions were then analyzed using NIH ImageJ software. Each region of interest was manually selected according to a mouse brain atlas [18]. To allow a more accurate assessment of staining, photographs were analyzed using a standardized background subtraction and the threshold function in ImageJ. For regions in which cell bodies were present in the absence of fibers (i.e. cortical regions), semi-quantitation represents the relative density of soma in the region of interest. For all other regions, the semi-quantitation of fiber density is presented. As counting of cell bodies in densely stained regions may be unreliable, cell bodies in these areas are described in Section 3. Fiber density was ranked on a scale of highest/maximum (++++) to low (+) density, as well as few to no (+/−) or no fibers present at all (0), as reported in Table 1.

3. Results

Semi-quantitative comparisons of NPY-ir in each meadow voles and prairie voles are presented in Table 1, with representative photomicrographs in Fig. 1. The cell types and distribution of both cells and fibers in these vole species are consistent with previous reports in other rodent species. Numerous large (12–16 μm in diameter) cell bodies were apparent in cortical areas, particularly in motor and somatosensory cortices (Figs. 2 and 3a). Perikarya were observed in layers II through VI, with layer V having a slightly higher density than all other layers. Cells were almost exclusively multipolar or bipolar.

Cell bodies were also observed in a number of subcortical regions. In the intermediate region of the lateral septum (LS), cells of moderate size (10 μm diameter) were rare but observed in some tissue. However, the presence of perikarya in the LS was an exception rather than typical for this region. Perikarya were also observed throughout the striatum, although cell types differed between the dorsal striatum and nucleus accumbens (NAc). In the dorsal striatum, there was a uniform distribution of low to moderately stained cells. These cells ranged from small to medium size (7–12 μm diameter), lacked uniform orientation, and processes were not clearly observed. The NAc was characterized by darkly stained cells of moderate size (8 μm diameter); Fig. 3b with multipolar processes, and were observed throughout the structure although more densely in the shell region.

Small, darkly stained cell bodies (2.5–5 μm diameter) were observed in a number of regions including the paraventricular nucleus of the hypothalamus (PVN; Fig. 3e), arcuate nucleus, paraventricular thalamic nucleus (PV thal), and intergeniculate leaflet (IGL). Each of these regions were also characterized by very high fiber density, which likewise makes an accurate count of cell density unreliable (see Fig. 3e).

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