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Brain mast cells are influenced by chemosensory cues associated with estrus induction in female prairie voles (*Microtus ochrogaster*)

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Abstract

Historically, the brain has been viewed as protected from the infiltration of peripheral hematopoietic cells by the blood-brain barrier. However, numerous immune cell types have been found in the central nervous system (CNS). Mast cells, granulocytic immune cells, are found in the CNS of birds and mammals and their numbers and location are influenced by both extrinsic and intrinsic factors, including reproductive behavior and endocrine status. The present study used female prairie voles (*Microtus ochrogaster*) to investigate the interactions between brain mast cells and stimuli associated with estrus induction. Unlike spontaneous ovulators such as rats and mice, female prairie voles are induced into estrus by chemosensory stimuli present in conspecific male urine. Prior to estrus induction, female voles have undetectable concentrations of estrogen that rise rapidly following exposure to a male or male urine. In the first experiment, we examined whether mast cells may be influenced by estrus induction. Female voles exposed to conspecific male urine had increased numbers of mast cells in the main olfactory bulbs and epithalamus (medial habenula), but not the thalamus or median eminence, relative to control groups. Next, to determine if this mast cell increase was the result of elevated estrogen concentrations, female voles were injected with estradiol or vehicle and brain mast cell numbers analyzed. No differences in brain mast cell numbers were observed between estradiol-injected and control females in any brain area investigated. Together, these results lend further support to the contention that mast cell numbers and/or distribution can be influenced by reproductively relevant stimuli and underscore the utility of this vole model for delineating the function of brain mast cells.

Keywords

Immune; Neuroendocrine; Estrus; Rodent; Hormone; Estrogen

Introduction

For organisms to function optimally, the endocrine and immune systems must be maintained within narrow operating limits. To accomplish this goal, complex reciprocal interactions exist among the nervous, endocrine, and immune systems (Ader et al., 2001; Webster et al., 2002). For example, both neural and endocrine factors have been shown to affect a variety of immune parameters (Ader et al., 2001). In addition, numerous brain regions have receptors for cytokines (diffusible factors secreted by immune cells) and cytokine administration can have marked effects on brain electrical activity (Besedovsky and Del Rey, 1996; Xia et al., 1999). Likewise, lesions or stimulation of specific brain sites can affect immune function (Ho et al., 1995; Schlesinger et al., 1995). Receptors for cytokines have also been identified on numerous endocrine glands (e.g., pituitary, thyroid, pancreas, and gonads), allowing the immune system to affect hormone production and secretion directly (Besedovsky and Del Rey, 1996). Hormone receptors have been identified on immune cells, and fluctuations in hormone secretion can modulate immune function (Elenkov and Chrousos, 2002; Eskandari and Sternberg, 2002; Martin, 2000). Thus, interactions among the nervous, endocrine, and immune systems act to modulate and fine-tune one another, particularly when one system is being preferentially activated.

Mast cells are granulocytic immune cells found peripherally in mucosal and serosal tissues, as well as in the CNS, often in close apposition to neurons and blood vessels (Dimitriadou et al., 1990; Johnson et al., Seeldrayers, 1991; Johnson and Krenger, 1992). Mast cells play an important role in innate (nonspecific) immune responses and are capable of secreting numerous substances, including neuro-transmitters, biogenic amines, and proteoglycans (Galli, 1990; Lambracht-Hall et al., 1990), and they degranulate in response to immunoglobulin E (IgE) and antigen stimulation (reviewed in Johnson and Krenger, 1992). A number of other factors can act on mast cells to cause the release of these mediators, including neurotransmitters and steroid hormones (Johnson and Krenger, 1992; Arvy, 1955; Befus, 1990; Vliagoftis et al., 1990). Mast cells are highly “plastic” and can change secretory properties depending on their environment (Kitamura et al., 1987a, 1987b), making them ideal for delivering biologically active substances to discrete regions in the brain and body on demand. The observation that mast cells are mobile immune cells located within both the CNS and periphery, coupled with evidence that steroid hormones and neurotransmitters can act on mast cells to modulate the release of their contents, suggests that mast cells may represent an important locus for neuroendocrine-immune interactions (Silver et al., 1996).

Although their precise role remains elusive, several studies have established that brain mast cells are influenced by reproductive behavior and endocrine status. In the medial habenula of ring doves (*Streptopelia risoria*), mast cells exhibit a marked increase in number and activation in response to sex behavior or sex steroids (Zhuang et al., 1993; Silver et al., 1993; Wilhelm et al., 2000). Mast cells are almost completely absent in the brains of long-term castrated males (Zhuang et al., 1993). In male mice, mast cells increase in discrete thalamic nuclei following mating and cohabitation with a female conspecific (Yang et al., 1999). As in male mice, mast cells increase in sensory, motor, and limbic thalamic regions in male rats following prolonged pairing with an estrogen-progesterone-primed female (Price, 1995; Asarian et al., 2002). Additionally, male Syrian hamsters housed in long day lengths or exposed to conspecific vaginal secretions display elevated brain mast cell numbers (Novak et al., 1994; Novak and Nunez, 1995).

Female prairie voles (*Microtus ochrogaster*) represent an ideal model system to study the interaction between reproductive events and brain mast cells because the onset of the stimulus initiating the cascade of endocrine events required to induce estrus can be strictly

controlled. Unlike spontaneous ovulators such as rats and mice, female voles do not exhibit estrous cycles and related cyclical hormonal changes. Instead, female voles are induced into estrus by exposure to male conspecifics due to a pheromone found in male urine (Richmond and Conaway, 1969; Richmond and Stehn, 1976; Carter et al., 1980). Isolated females have undetectable circulating concentrations of luteinizing hormone and estradiol that increase rapidly following exposure to a male (Cohens-Parsons and Carter, 1987). The unique endocrine profile of female voles, as well as the fact that their reproductive endocrine status can be strictly controlled, affords the opportunity to investigate the interactions among mast cells, reproductive events, and the endocrine system. As a result, the present studies investigated whether brain mast cells change in response to estrus induction and resulting hormonal changes. In addition, to determine whether any changes in mast cells following estrus induction are the result of increased estradiol concentrations, the present experiments also examined the effects of exogenous estradiol on brain mast cell numbers in isolated females.

Materials and methods

Experiment 1

Animals—Forty-one adult (>60 days of age) female prairie voles (*M. ochrogaster*) were obtained from our laboratory breeding colony. Animals were housed individually in polypropylene cages (28 × 17 × 12 cm) in a 16L:8D photoperiod from birth to the end of the study. The vivaria were temperature controlled (20 ± 2°C) and maintained with relative humidity of 50 ± 5%. Laboratory chow (Agway ProLab 2000) and tap water were available ad libitum for the duration of the experiment. All procedures were approved by the Johns Hopkins University Animal Care and Use Committee.

Housing conditions—At 70 days of age, animals were exposed to one of four conditions. In the first condition, a single drop of conspecific male urine was placed onto the nares of females ($n = 15$), daily, for 6 consecutive days. Three control groups were also formed. One group of females received a drop of water ($n = 9$) for 6 consecutive days. Another group of females ($n = 8$) received a drop of 5% nonfat dry milk (Carnation, Los Angeles, CA) for 6 consecutive days. Nonfat dry milk was used instead of saline because it provided chemosensory stimulation. The last group was unmanipulated in its home cage ($n = 9$).

Brain removal and acidic toluidine blue staining—On day 7, animals were injected with a lethal dose of sodium pentobarbital and perfused through the heart with 50 ml of saline followed by a 250-ml 4% paraformaldehyde solution. Brains were then postfixed for 3 h, cryoprotected in a 20% sucrose solution overnight, and sectioned at 40 μm in the coronal plane using a cryostat and the sections were collected into 0.1 M phosphate buffered saline (PBS, pH 7.4). Alternate sections from the region of the olfactory bulb to the caudal aspect of the median eminence were mounted onto gelatin-coated slides, stained with acidic toluidine blue (pH 2.0), allowed to air-dry overnight, and dehydrated in a graded series of ethanol solutions (30, 70, 95, 100%), and coverslips were applied. Mast cells display violet metachromasia when stained with acidic toluidine blue and surrounding neurons and glia appear light blue.

Immunohistochemistry for histamine—As one of the mediators found in mast cells is histamine, three additional animals were treated as described above to provide converging evidence that mast cells were being counted. On the seventh day of treatment, animals were injected with a lethal dose of sodium pentobarbital and perfused through the heart with 250 ml of freshly prepared 4% 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (Sigma) in 0.1 M PB (pH 7.4), as required for the demonstration of histamine immunoreactivity. Brains were

cut on a cryostat at 60 μm in the coronal plane. Localization of histamine immunoreactivity was accomplished as previously described (Silverman et al., 1994). Briefly, sections were incubated in 0.5% hydrogen peroxide to remove endogenous peroxidase activity. Following preincubation in blocking solution (10% normal goat serum, Vector Laboratories), sections were incubated for 48 h at 4°C in rabbit antihistamine (Incstar, Stillwater, CA) diluted 1:2000. Sections were then incubated in biotinylated goat antirabbit IgG (1:250, Vector Laboratories) followed by avidin-biotin-HRP complex (Vector Laboratories). HRP label was demonstrated using 0.04% diaminobenzidine (Polysciences Inc.) in 0.1 M PBS as the chromogen with 0.1% hydrogen peroxide as the substrate. Sections were dehydrated in a graded series of ethanol solutions (30, 70, 95, 100%) and cleared in histoclear (Fisher), and coverslips were applied.

Electron microscopy—Sections through the medial habenula were cut on a vibratome at 50 μm and the nucleus was microdissected. The tissue was treated with 2% OsO_4 in 0.9% saline/1.5% $\text{K}_3\text{Fe}(\text{CN})_6$, and then dehydrated in a graded series of ethanol solutions and propylene oxide and embedded in Epon 812. Ultrathin sections were collected on formvar-coated slot hole grids and viewed with a JEOL 1200EX microscope.

Data analysis and cell counting—Slides were examined under bright-field illumination on a Nikon Optiphot microscope. The distribution of mast cells was mapped onto a mouse brain atlas (Slotnick and Leonard, 1975). The number of mast cells was counted by two observers, each of whom was unaware of the experimental conditions to which the animals were exposed. The final mast cell numbers were averaged within each experimental condition. For cell measurements, all analyses were performed under $\times 400$ magnification. Cells were measured by sending microscopic fields of view onto a power MacIntosh 7600 computer using a Sanyo CCD video camera (Model No. VCC-3972) connected to a Nikon Optiphot microscope. Cells were outlined and the two-dimensional area was calculated using NIH Image 1.61.

Cells were counted in every brain section comprising the regions being quantified. For each brain, total regional cell counts represented the total number of cells from all sections comprising a particular brain region. Because the data violated the homogeneity of variance assumption, data were analyzed using a Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks. Pairwise comparisons were conducted using the Tukey-HSD test and planned comparisons where appropriate (Keppel, 1991). Comparisons were considered statistically significant when $P < 0.05$.

Experiment 2

Experimental treatment and tissue processing—Animals were obtained from our colony as described above. Female voles were injected for 2 consecutive days with either 6 μg of 17 beta estradiol ($n = 8$) or the oil vehicle alone ($n = 8$). Pilot data in our lab have shown that this treatment results in uterine masses equivalent to animals exposed to 6 days of male urine treatment. On day 6, animals were injected with a lethal dose of sodium pentobarbital and perfused through the heart with 50 ml saline followed by a 250-ml 4% paraformaldehyde solution. The ovaries and uterine horns were removed from the animals, cleaned of fat and connective tissue, and weighed to the nearest milligram. Brains were prepared and stained with acidic toluidine blue as described for Experiment 1.

Data analysis and cell counting—All cell counts were performed as described in Experiment 1. Data were analyzed using Student's unpaired t tests. Results were considered statistically significant if $P < 0.05$.

Results

Experiment 1

Brain mast cells were examined in the olfactory bulbs, medial habenula, hippocampus, thalamus, and median eminence of all animals. Very few mast cells were observed in the hippocampus, and these data were not analyzed. In all the brain areas investigated, mast cells displayed violet metachromasia and clearly defined granules. Mast cells were easily distinguishable from surrounding neurons; neuronal cell bodies and glia stained light blue, whereas mast cells stained violet (Fig. 1). The mean cell diameter of the observed mast cells was $8.26 \pm 0.26 \mu\text{m}$ and the mean cell area was $266.29 \pm 1.77 \mu\text{m}^2$. Immunohistochemistry for histamine revealed cells with a similar, rounded morphology in a distribution comparable to that seen with toluidine blue staining (Fig. 1). At the ultrastructural level, the mast cells had a heterochromatic nucleus and the cytoplasm was filled with homogeneously dense secretory granules, properties characteristic of mast cells at the ultrastructural level (Fig. 2).

The number of mast cells stained with acidic toluidine blue was affected by the experimental treatment to which the animals were exposed. Specifically, animals exposed to urine for 6 days had increased numbers of mast cells in the main olfactory bulb and medial habenula relative to control groups exposed to water, milk, or animals that were not manipulated ($P < 0.05$ in each case; Figs. 3 and 4). In the olfactory bulb, exposure to milk also increased mast cell numbers above animals exposed to water ($P < 0.05$; Figs. 3 and 4). Mast cell numbers were unaffected by any experimental treatment in the thalamus ($P > 0.05$; water = 115.25 ± 50.11 , milk = 168.25 ± 44.80 , urine = 162.5 ± 120.59 , unmanipulated = 73.66 ± 48.01) and median eminence ($P > 0.05$; water = 9.0 ± 4.83 , milk = 2.5 ± 1.04 , urine = 6.5 ± 2.54 , unmanipulated = 18.5 ± 18.3) (Figs. 3 and 4). Because a previous study had shown that increases in brain mast cells following cohabitation in rats are specific to a particular rostrocaudal extent of the thalamus (Asarian et al., 2002), the distribution of thalamic mast cells was investigated. Mast cells were distributed uniformly throughout the rostrocaudal extent of the thalamus across groups, with most cells being observed in the paraventricular thalamic region directly below the medial habenula. Some animals exhibited cells more dispersed throughout the thalamus, often in close appositions to blood vessels (Fig. 5).

Mast cell numbers in mammalian species often exhibit high interindividual variability. Therefore, a regression coefficient was calculated using the Pearson product-moment correlation to compare brain areas to determine if this variability was associated with all populations of mast cells within an individual. Animals with high numbers of cells in the olfactory bulbs had high numbers of cells in the medial habenula ($r = 0.728$, $P < 0.0001$). High numbers of mast cells in the thalamus also corresponded with high numbers of mast cells in the median eminence ($r = 0.898$, $P < 0.0001$). Because factors other than urine application may induce female voles into estrus (Richmond and Conway, 1969), animals exposed to urine, water, or milk (manipulated) were compared to unmanipulated animals. Manipulated animals exhibited increased mast cell numbers in main olfactory bulbs, medial habenula, and perihippocampal areas compared to animals that were not treated ($P < 0.05$; data not shown). However, manipulated animals did not display significantly increased mast cell numbers in comparison to unmanipulated controls for the thalamus and median eminence brain regions ($P > 0.05$; data not shown).

Experiment 2

Brain mast cells numbers were analyzed in the olfactory bulb, medial habenula, thalamus, and median eminence. Cells exhibited staining characteristics and morphology identical to mast cells in Experiment 1. To confirm that estrogen treatment affected uterine mass to a similar extent as previous studies of estrus induction that used male urine, uterine weights

were examined. Uterine mass was significantly increased in female voles injected with estradiol relative to voles injected with the vehicle ($P < 0.05$; vehicle = 40.3 ± 6.02 mg, estradiol = 65.4 ± 4.46 mg), whereas body mass was unaffected ($P > 0.05$; vehicle = 34.04 ± 1.62 g, estradiol = 31.67 ± 1.91 g). However, unlike urine treatment in Experiment 1, mast cell numbers (or size of the mast cell population) in the olfactory bulbs and medial habenula were unaffected by the estrogen treatment ($P > 0.05$; Table 1). Similarly, mast cell numbers in the thalamus and median eminence were unaffected by estrogen treatment ($P > 0.05$). In agreement with the results of Experiment 1, animals with higher numbers of mast cells in one region of the brain tended to have higher numbers of mast cells in other brain regions.

Discussion

In the present study, female voles exposed to conspecific male urine exhibit a marked increase in the number of mast cells observed in the olfactory bulbs and medial habenula. Unlike results obtained in ring doves (Wilhelm et al., 2000; Zhuang et al., 1996), hormone treatment did not affect mast cell numbers. Although mast cells were abundant in the thalamus and median eminence, their numbers were not influenced by reproductive condition or hormone treatment, suggesting that the effects are specific to the olfactory system and medial habenula. Together, these results add to the growing database of species that exhibit increases in brain mast cells in response to reproductively relevant stimuli. Likewise, these findings suggest that female prairie voles represent an excellent model system to identify the specific mechanisms and functional significance of alterations in brain mast cell numbers and location.

The evidence for mast cells in the brains of birds and rodents is vast, although the precise function of these immune cells in the brain remains unknown (e.g., Silver et al., 1992, 1993; Silverman et al., 1994, 1995; Wilhelm et al., 2000; Zhuang et al., 1993; Dimitriadou et al., 1990; Johnson et al., 1991; Johnson and Krenger 1992). In the present study, two independent markers revealed the presence of mast cells in the brains of female prairie voles. First, acidic toluidine blue staining revealed nonneuronal cells that displayed violet metachromasia. Metachromasia to aniline dyes is a histochemical property characteristic of mast cells, thereby allowing one to distinguish mast cells from surrounding neurons. Second, immunohistochemistry for histamine, one of the primary mediators found in mast cells, revealed cells with a similar rounded morphology and a distribution pattern seen with toluidine blue (see Fig. 1). Because histamine is one of the major components of mast cell granules in most mammals (Siraganian, 1988), histamine immunohistochemistry provides a reliable marker for mast cells in the central nervous system. Finally, at the ultrastructural level, it was confirmed that the cells investigated at the light microscopic level were, indeed, mast cells.

It is noteworthy that among the regions that exhibit alterations in mast cell numbers is the medial habenula. The present results are reminiscent of the reports that mast cells increase in the medial habenula of male ring doves following 2 h of courtship (Silver et al., 1992, 1993; Silverman et al., 1994; Zhuang et al., 1993). This finding indicates that the medial habenula may be an important brain region in which mast cells congregate and possibly secrete their contents (Silver et al., 1996). The main afferent into the medial habenula comes from the septal area via the stria medullaris, whereas the primary efferent from the habenula terminates in the interpeduncular nucleus (reviewed in Sutherland, 1982). Thus, the habenula allows communication between the limbic forebrain and the midbrain. Additionally, striatal efferents terminate in the medial habenula, suggesting that the habenula may represent an important site of convergence between the limbic and striatal systems. Several lines of evidence also suggest that the habenula is involved in female mating behavior. For example, bilateral habenula lesions reduce receptive behavior of

ovariectomized, estrogen-primed female rats (Modianos et al., 1974, 1975). Other studies have reported that the habenula may play an inhibitory role in reproductive behavior. In one study, electrical stimulation of the medial habenula immediately prior to or during testing reduced receptivity in ovariectomized female rats (Moss et al., 1974). Taken together, the habenular complex may play an important role in copulatory behavior and mast cells may act at this neural locus to influence reproduction.

Traditionally, the brain has been considered to be protected from the infiltration of immune cells by the blood- brain barrier. However, it is becoming increasingly clear that immune cells and their products (e.g., cytokines) are present in the brain. As mentioned previously, interactions between the immune and endocrine systems act to maintain both systems within fine operating limits. Brain mast cells are influenced by events that result in increased hormone secretion such as exposure to a mate or stimuli associated with a potential mate (Asarian et al., 2000; Novak et al., 1994; Novak and Nunez, 1995; Yang et al., 1995; Zhuang et al., 1996) and following stress (e.g., Theoharides et al., 1995). The fact that mast cells increase following activation of the endocrine system suggests that mast cells may migrate to the brain to act on the CNS to modulate endocrine function. Mast cells release a variety of neurochemicals that can act to modulate neuroendocrine cells (or their terminals in the median eminence) to alter hormone production/secretion (Benoist and Mathis, 2002), thereby lending support for this possibility.

Although the role of peripheral mast cells in allergic reactions and other immediate hypersensitivity responses has been well-studied, the role of brain mast cells remains a challenge. Mast cells release a variety of cytokines and chemokines that can have powerful effects on the CNS (reviewed in Benoist and Mathis, 2002; Besedovsky and Del Rey, 1996; Xia et al., 1999). In addition mast cells have secondary responses that activate other cells of the immune system, including T cells and B cells (reviewed in Benoist and Mathis, 2002). Finally, mast cells release the powerful vasodilator histamine, which could change local blood vessel permeability in the brain (Esposito et al., 2002; Zhuang et al., 1996). Thus, CNS changes in mast cells and local release of their mediators could lead to changes in endocrine function, immune function, or blood vessel permeability.

One recent line of evidence for a role of *brain* mast cells in immune responses comes from studies of peripheral lipopolysaccharide (LPS) injections with or without prior intracerebroventricular (ICV) exposure to the mast cell stabilizer, sodium cromoglycate (Nava and Caputi, 1999). LPS administration leads to adaptive sickness behaviors such as reduced food and water intake, fever, and reduced locomotion. All of these measures are antagonized by prior brain injections of cromoglycate sodium salt (Nava and Caputi, 1999), suggesting that these response are modulated by brain mast cells and their chemical mediators. Thus, mast cell reorganization in response to environmental stimuli signaling potential exposure to pathogens may result in local changes in neural function and blood vessel permeability necessary to rapidly modulate peripheral immune and endocrine responses.

In the present study, both male urine and milk produced an increase in olfactory bulb mast cells, although urine produced a greater increase than milk. This finding suggests that olfactory mast cells may increase in response to any strong olfactory stimulus, and urine may represent a stronger stimulus than milk. However, mast cells in the medial habenula were only affected by male urine. This finding suggests that, with regard to mast cell activation, fine discrimination of reproductively relevant and nonrelevant ol-factory stimuli may not be processed in the olfactory bulbs but may require further processing within the epithalamus or in limbic areas projecting to this brain region. Future studies comparing the effects of other urine stimuli, such as female urine and castrated male urine (both incapable

of inducing estrus), will allow the determination of whether mast cells are affected by olfactory stimuli, chemosensory stimuli capable of inducing estrus, or any chemosensory stimuli signaling interactions with conspecifics.

In the present study, although estrus induction was associated with alterations in brain mast cells, estradiol exposure did not affect brain mast cell numbers or location. This finding suggests that either brain mast cells are not influenced by changes in estrogen associated with estrus induction (or chemosensory stimulation) or the treatment in the present experiment did not effectively mimic the endogenous pattern of hormone increase following estrus induction. It is possible that estrus induction is associated with a prolonged elevation of estradiol concentrations, and continued injections in the present study may have led to brain mast cell changes mirroring those following conspecific urine exposure.

The experiments presented herein represent an initial attempt to understand the role of mast cells in the interactions among the nervous, endocrine, and immune systems. The results establish that mast cells can infiltrate into the neuropil of the brains of female prairie voles following reproductively relevant stimuli. The precise role of brain mast cells following behavioral and pheromonal manipulations remains to be determined. Female prairie voles represent an ideal model for beginning to clarify the complex interactions among the neural, endocrine, and immune systems using a species in which behavioral and pheromonal events associated with reproductive endocrinology can be discretely manipulated.

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Fig. 1.

Representative mast cells from the brains of female prairie voles exposed to urine for 6 consecutive days. Mast cells were identified using acidic toluidine blue (TB) staining (top, A) or histamine immunohistochemistry (middle and bottom, B and C respectively). The cells in the top and bottom panels were located in the neuropil of the thalamus, whereas the cells in the middle panel were located in the neuropil of the medial habenula (i.e., epithalamus). BV, blood vessel.

Fig. 2.

The granule-filled cytoplasm of a mast cell is shown in this low-power electron micrograph. The nucleus of the cell is not in this section. The cell is located between two capillaries in the medial habenula of a female vole exposed to urine. Arrows point to the cytoplasm of the endothelial cells. A nucleus of a pericyte (P), a cell frequently found just interior to the endothelia is also visible. Given its position, secretion by the mast cell could influence the properties of the endothelial cells and the neuropil of the CNS. Scale bar = 1 μ m.

Fig. 3. Representative photomicrographs of the olfactory bulbs (top, A-D) and medial habenula (bottom, E-H) of female voles exposed to water, milk, or urine for 6 consecutive days, or animals that were left unmanipulated in their home cage. Arrows heads point to all mast cells in a given section. Larger arrows in the olfactory bulbs point to cells that are shown at high power in the inserts.

Fig. 4. Mean ($\pm$ SEM) number of mast cells in the olfactory bulbs and habenula of female voles exposed to water, milk, or urine for 6 consecutive days, or animals that were not manipulated. *Significantly greater than all other groups ($P < 0.05$). †Significantly greater than animals exposed to water ($P < 0.05$).

Fig. 5.

Mean ($\pm$ SEM) mast cell counts through the rostrocaudal extent of the thalamus of female prairie voles exposed to water ($n = 9$), milk ($n = 8$), urine ($n = 15$), or animals that were unmanipulated ($n = 9$). For the purposes of presentation, each data point represents the mean ($\pm$ SEM) across three brain sections for each animal.

Table 1

Mean ($\pm$ SEM) number of mast cells in the olfactory bulbs, habenula, thalamus, and median eminence of female voles that were injected either with proestrus concentrations of estradiol or given an oil injection

Brain region	Oil	Estradiol
Olfactory bulbs	89.20 (45.91)	75.14 (26.68)
Habenula	49.20 (17.15)	46.63 (11.43)
Thalamus	101.16 (34.24)	62.12 (31.98)
Median eminence	15.50 (7.23)	8.00 (3.19)