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Neuronal activation in the caudal brainstem associated with mating by voles

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Abstract

The expression of c-fos, a marker of neuronal activation, was examined in the gracile nucleus (GN) and nucleus of the solitary tract (NTS) after social interactions, including mating, between male and female prairie voles. In GN, mating, but not non-sexual interactions, induced similar significant increases in c-fos immunoreactivity in both males and females. The increased immunoreactivity was concentrated in medial and dorsal GN suggesting that expression was driven by stimulation of reproductive organs. In contrast, in NTS, mating-induced increases in c-fos expression occurred only in males. These results suggest that both GN and NTS comprise different functional components of mating circuitry and may contribute to pair bonding in monogamous voles.

Keywords: c-fos; Nucleus of the solitary tract; Gracile; Sensory; Social; *Microtus*; Pair bonding

Mating and its associated stimuli can produce long-term behavioral and endocrine changes. In female rats, vagino-cervical stimulation terminates behavioral estrous [12], induces maternal behavior [19], triggers the onset of twice-daily prolactin surges [7], and induces pseudopregnancy [1]. In monogamous voles, mating facilitates pair bonding [17]. Studies examining mating-associated c-fos expression (cf. Ref. [13]) have focused primarily on the forebrain. It has recently been found that, in the caudal brainstem, neuronal activity in both the gracile nucleus (GN) and the nucleus of the solitary tract (NTS) is altered by stimulation of the vaginal canal and cervix [2,9]. Because these areas are stimulated during mating, it is possible that neurons in the GN and NTS are components of the brain circuitry by which mating produces long-term effects. Accordingly, we have examined c-fos expression in GN and NTS in response to mating in monogamous prairie voles (*Microtus ochrogaster*).

Subjects were of the F4 generation of a laboratory colony originating from Illinois. Pups were weaned at ~21 days of age and kept in same-sex sibling pairs until used in experiments. Voles were housed in plastic shoebox style cages (20 × 50 × 40 cm) with a 14L/10D photoperiod and ad libitum food and water. Subjects were sexually mature (~70 days old) at the time of the experiments.

Perfusion, c-fos immunocytochemical processing, and analysis of c-fos immunoreactivity were as previously described [16]. The primary antibody was rabbit anti-rat-c-fos (Santa Cruz) diluted 1:30,000 and the secondary antibody was biotinylated goat-anti-rabbit. Staining was visualized by reacting the tissue with 3,3′-diaminobenzidine hydrochloride and 0.04% nickel ammonium sulfate. c-fos immunoreactivity was assessed in the GN and NTS by investigators blind to the experimental group. The area postrema (AP) and dorsal motor nucleus of the vagus (DMV) were also assessed and used as controls for generalized activation. For each animal, every fourth 40 μm section (four to five sections per area per animal) through the rostral-caudal extent of the AP was analyzed and the mean number of c-fos immunoreactive (c-fos-ir) cells for each area was calculated.

Treatment with estradiol benzoate (EB, 0.1 μg/day for 3 days) induces sexual receptivity in most female prairie voles [18]. Because voles, unlike rats, do not experience estrous cycles, ovariectomy is not considered necessary to ensure consistent estrogen levels among females. Furthermore, voles do not require progesterone to induce sexual receptivity [6]. Sexually naive, EB-treated adult females were randomly assigned to one of two experimental groups.

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In one group, both individuals from same-sex sibling pairs ($n = 6$ for each sex) were transferred to new cages where they remained together for 3 h prior to sacrifice. In the second group, male/female pairings were set up in new cages. Despite exposure to a sexually receptive female, some male voles will not mate, thus cohabitation periods were videotaped (Panasonic time-lapse video recorder, 12:1 compression) and the videotapes were later analyzed to assess the presence or absence of mating. Individuals were assigned to unmated sub-groups ($n = 5$ of each sex) if mating did not occur, while those that mated were assigned to mated sub-groups ($n = 5$ of each sex). At the end of 3 h of cohabitation, both animals in each pair were sacrificed and the brainstems processed for immunocytochemistry. A two-way ANOVA (sex by treatment) was used to assess differences in the numbers of c-fos-ir nuclei. Within-sex differences were assessed using one-way ANOVA. Post hoc comparisons were made using Student–Newman–Keuls analysis.

c-fos-ir cells were found in the GN of prairie voles of both sexes (Fig. 1). c-fos-ir nuclei were most densely concentrated in the medial and, to a lesser extent, dorsal portions of the GN (Fig. 1). There was a significant group effect ($F_{2,27} = 19.0$, $P < 0.001$) but no effect of sex ($F_{1,27} = 0.1$, $P = 0.77$) and no interaction ($F_{2,27} = 0.03$, $P = 0.92$). Mated animals of either sex (Fig. 2A) displayed more c-fos-ir cells than did baseline controls or unmated animals exposed to a stranger of the opposite sex (males, $F_{2,14} = 11.9$, $P < 0.01$; females, $F_{2,12} = 7.7$, $P < 0.01$). Mating-associated c-fos induction was sex-specific in the NTS (Fig. 2B). One-way ANOVA showed a significant group effect in males ($F_{2,13} = 5.9$, $P < 0.02$) with mated males having higher numbers of c-fos-ir nuclei than either males that remained with their siblings or males exposed to females but that did not mate. Females showed no changes in c-fos-ir in NTS regardless of the social situation ($P = 0.94$). No group effects were found in the AP ($P = 0.46$) or in the DMV ($P = 0.66$). Thus, mating-associated activation of the GN and NTS was not a result of generalized activation.

The findings here of mating-associated increases in c-fos expression in GN are consistent with results of previous studies in rats showing that GN neurons process sensory information from internal pelvic organs [2]. Because these previous studies also showed that responses of GN neurons to pelvic stimulation change across the rat's estrous cycle [2], it is possible that c-fos expression changes observed here were influenced by the vole's estrogen and progesterone levels. Due to their reflex ovulation, voles do not experience cyclic fluctuations in progesterone, and functional corpora lutea are not observed until at least 10 h after mating [3]. Thus, c-fos expression in GN was unlikely to be affected by progesterone. Likewise, endogenous estrogen is unlikely to have affected c-fos expression. Although

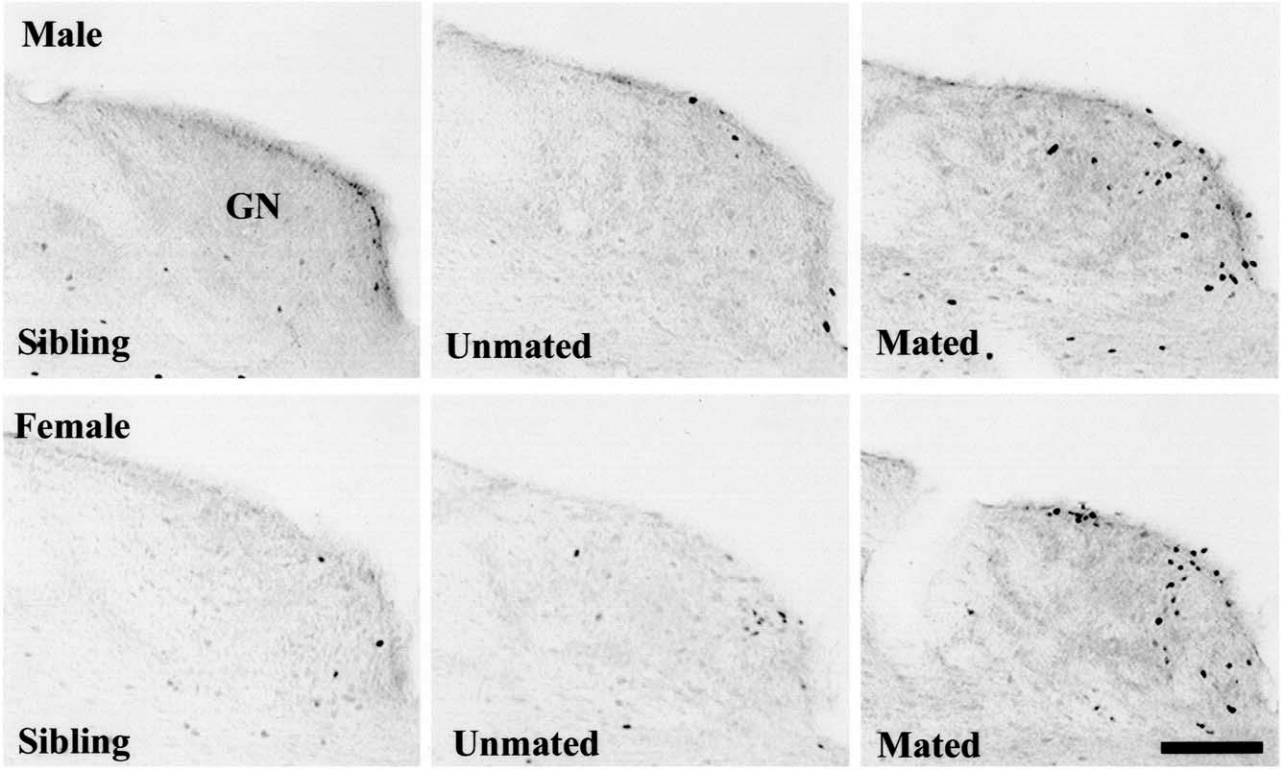

Fig. 1. Micrographs showing fos-ir cells in the GN in male (top panels) and female (bottom panels) prairie voles after three hours of exposure to a same-sex sibling cagemate (Sibling), an unfamiliar opposite sex conspecific without mating (Unmated), or an unfamiliar opposite sex conspecific with mating (Mated). All females were EB treated. Scale bar, 100 μm.

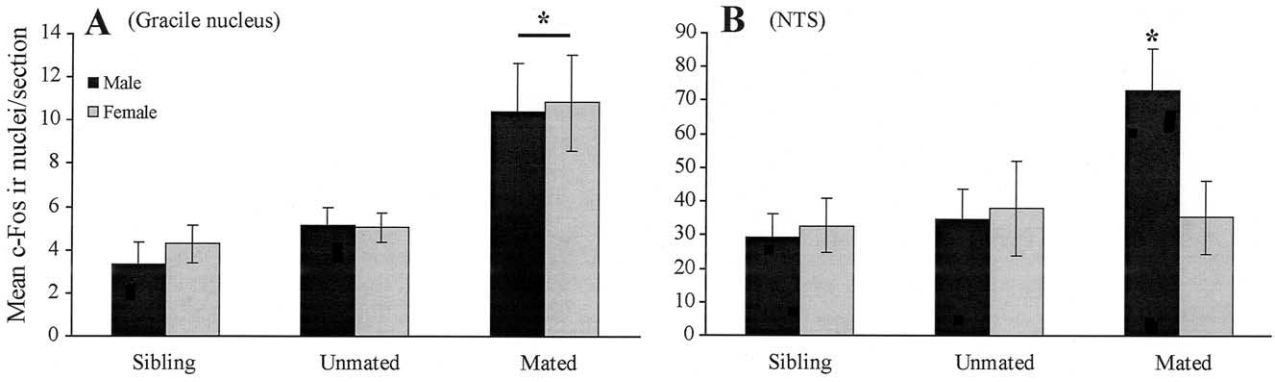

Fig. 2. Numbers of fos-ir cells in the GN (A) and NTS (B) of prairie voles after 3 h of exposure to either a same-sex sibling cagemate (Sibling), an unfamiliar opposite sex conspecific without mating (Unmated), or an unfamiliar opposite sex conspecific with mating (Mated). Data are means ± SE. * indicates significantly different from either sibling or unmated groups.

exposure to an adult male increases circulating levels of estrogen in female voles, the 3 h exposure here was too short to do so [5]. It remains possible, however, that the EB treatments used to induce sexual receptivity may have affected the magnitude of c-fos induction in the females.

It is uncertain what aspects of mating increased c-fos expression in GN. In female rats, GN neurons respond to brushing the skin of the perineum, tail, or flank, as well as to stimulation of the vagina and cervix [2]. Because all of these body areas are likely to be stimulated during mating in female voles, any or all of the stimuli could have affected the c-fos expression in GN of females here. On the other hand, the fact that similar changes occurred in males and females and that different portions of hindlimb skin are stimulated during mating in males and females suggests that the changes in both sexes were most likely the result of genital stimulation. In further support of this conclusion is the observation here that the c-fos-ir cells after mating were concentrated in the dorsal and medial part of the GN at the level of the obex. Hubscher [8] showed that neurons responsive to stimulation of the vagina and cervix were most densely located in this region.

Mating-associated increases in c-fos expression in the NTS were not unexpected because previous studies have shown that stimulation of pelvic viscera activates neurons in the part of the NTS studied here [9]. However, an explanation for the apparent sex-specificity in the responses is not readily apparent. Hubscher and Berkley [9] suggested that the pelvic visceral information processed by neurons in the NTS and GN likely serves different behavioral and/or physiological functions. A report [4] that cardiovascular responses and analgesic effects of vagino-cervical stimulation arise from different mechanisms is consistent with this idea. However, because it is likely that both sexes experience both cardiovascular changes and analgesic effects during mating (cf. Refs. [4,11]), it is unlikely that sex differences in these aspects of mating explain the present results. Alternatively, since the tissues stimulated during copulation in both sexes are innervated by several peripheral nerves, including the vagus [10], the differential activation in males and females may represent sex-specific relative contributions of the different nerves. Finally, there may be sex-specific patterns in the relative amounts of excitatory vs. inhibitory inputs to the NTS. In any case, the sex-specific responses to mating in NTS deserve further examination.

As mentioned above, mating facilitates pair bonding in prairie voles. Thus, it is possible that the activity of GN and/or NTS neurons contributes to the formation of pair bonds. Of particular relevance are the forebrain projections of NTS neurons. Vasopressin (AVP) and oxytocin (OT) have been strongly implicated in pair bonding [20], and NTS can influence the paraventricular and supraoptic nuclei which are important sites for the synthesis and release of these peptides [14]. Of possibly greater significance, Sim and Joseph [15] showed that cells in the same portion of the NTS as was examined in the present study project to the medial pre-optic area, bed nucleus of the stria terminalis, and medial amygdala, all of which have also been implicated in pair bonding in voles [16]. The significance of the sex differences in c-fos activation in NTS, however, remains unclear. One possibility is that activation of NTS in males suppresses what would otherwise be a propensity for the males not to form pair bonds. Such a possibility could be examined by studying mating-induced c-fos expression in the NTS of other species of voles that do not form pair bonds.

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