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Research report

Sexual and social experience is associated with different patterns of behavior and neural activation in male prairie voles

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Abstract

Monogamous prairie voles (*Microtus ochrogaster*) show mating-induced aggression towards conspecific strangers. This behavior is both selective and enduring. The present study was designed to investigate the behavioral conditions for the emergence of selective aggression (by varying prior experience with a female and identity of intruders) and the limbic activation in response to an intruder (by mapping regional staining for c-*fos*) in male prairie voles. In a first experiment, males that mated with a female for 24 h exhibited aggression towards a male intruder and had more Fos-immunoreactive (Fos-ir) cells in the medial amygdala (AMYGme) and medial preoptic area (MPO) relative to males that cohabited with a female without mating or that had no prior exposure to a female. Cohabited males did not become aggressive. However, these males along with mated males had an increased number of Fos-ir cells in the lateral septum (LS) and the bed nucleus of the stria terminalis (BST) relative to males without prior exposure to a female. In a second experiment, mated males exhibited more offensive aggression to a male intruder but more defensive aggression to a female intruder. Both types of aggression, however, induced an increase in the number of Fos-ir cells in the AMYGme. In addition, Fos-ir staining in the BST was induced selectively in response to a male intruder and a similar trend was found in the LS. Exposure to a male or female intruder did not increase Fos-ir staining in the MPO. Taken together, our data suggest the neural substrates activated by social/sexual activity and involved in response to intruders. The AMYGme was involved in processing intruder-related cues and/or in the regulation of aggressive response to both male and female intruders. The BST and LS were modulated by social experience with a female (mating or cohabitation) and were responsive to male-related cues even in the absence of aggression. Finally, the MPO was activated at different magnitudes by social or sexual experience but did not respond to intruder-related cues as measured by Fos-ir. © 1997 Elsevier Science B.V.

Keywords: Monogamy; Aggression; Amygdala; Lateral septum

1. Introduction

The prairie vole (*Microtus ochrogaster*) is a monogamous rodent that forms pair bonds after mating. Prior to mating, the male vole is a highly affiliative member of a multi-generational family group. Within 24 h of mating, the male appears to undergo a behavioral transformation. In contrast to the virgin male that grooms and huddles with strangers, the sexually-experienced male attacks conspecific intruders and exhibits affiliative behaviors only towards his mate [9,10,20,25,39,45]. Both selective aggression and affiliation appear to be dependent on mating as cohabitation with a female without mating does not induce such behaviors [45]. In addition, these behaviors are enduring: even in the absence of the female mate, the male continues to show aggression for at least one week [25,45]. As non-monogamous voles fail to show these behaviors after mating, the emergence of selective aggression and affiliation in prairie voles appears to reflect the development of a pair bond associated with a monogamous life strategy [25,39]. Indeed, the selective aggression from an ethological perspective probably denotes mate and territory guarding [9].

Although the behavioral consequences of mating have been studied in prairie voles, the neural mechanisms underlying these behaviors are still unknown. The current study was designed to investigate regional brain activation associated with pair bonding by focusing on mating-induced aggression in male prairie voles. We used the resident–intruder paradigm to study male aggression and Fos immunoreactivity (Fos-ir) to examine regional neural activation. Fos is a protein product of the immediate early gene c-*fos* that is rapidly induced following sensory stimu-

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lation and can be used as a marker of multisynaptic pathways [24,40]. In the current study, by comparing regional Fos-ir induction in mated and non-mated males during an intruder test, we attempted to detect activation of different pathways representing alternative processing of the sensory stimulus representing either 'friend' or 'foe'.

Which brain areas are most likely to be involved in mating induced aggression in prairie voles? We focused on the amygdala (AMYG), bed nucleus of the stria terminalis (BST), lateral septum (LS), and medial preoptic area (MPO). All these limbic areas have been previously implicated in the regulation of social behaviors in rodents [5,16,17,23,26,32,33,35,37]. In prairie voles, these areas have been previously implicated in affiliative and parental behaviors [28,29,42]. In a first experiment, we examined inter-male aggression and Fos-ir staining in male voles that were either mated, housed with a female without mating ('cohabitation'), or housed alone without prior experience with a female. Fos-ir staining was also examined in males that had neither female experience nor a male intruder test (control). The purpose of this experiment was to distinguish brain areas that were activated specifically by mating-induced aggression from the areas that were activated by non-specific aspects of social exposure to an intruder. In a second experiment, we examined aggression and Fos-ir induction in mated males that were tested with either a male intruder, a female intruder or a female partner, or that did not receive an intruder test (control). We hypothesized that patterns of regional Fos-ir staining evident in those animals exhibiting aggression would indicate differential information processing or motor responses as a result of exposure to different intruders. In addition, differences in Fos-ir staining between mated and cohabited groups may also represent different effects of sexual or social experience on neural activation.

2. Materials and methods

2.1. Subjects

Subjects were initially sexually naive male prairie voles (*Microtus ochrogaster*) that were the F1 generation of a laboratory breeding colony started with field captured animals. After weaning (21 days old), subjects were housed in same sex sibling pairs in plastic cages ($20 \times 25 \times 40$ cm) which contained cedar chip as bedding. Water and food were provided ad libitum. All voles were maintained on a 14:10 h light/dark photoperiod with lights on at 07.00 h. The temperature in the colony room was about 20°C. At 60–90 days of age, subjects were separated and housed individually for one week before the experiment.

2.2. Treatment procedures

In a first experiment, subjects were divided into four groups. In group 1 ($n = 6$), subjects were mated with a sexually receptive female for 24 h. To ensure that the female would be sexually receptive, we injected the adult female with 0.05 μg estradiol benzoate for 2 days which successfully induced the female into estrus [45]. Male prairie voles generally begin mating within 3 h of being placed with a sexually receptive female and continue to copulate every 45–60 min throughout the subsequent 24 h [11]. The female was removed following 24 h mating and the male subject was housed alone for 24 h to reduce the effects of female exposure on Fos activation. After that, subjects received a 6-min male intruder test (see below). In group 2 ($n = 4$), subjects were housed with a female for 24 h without mating, housed alone for 24 h after the female was removed, and then received a male intruder test. To preclude mating in this group, females were ovariectomized. In group 3 ($n = 5$), subjects were housed alone without female exposure for 2 days (parallel to group 1 and 2) and then received a male intruder test. In group 4 (control, $n = 6$), subjects had neither exposure to a female nor male intruder test. These subjects, however, were also videotaped for 6 min (equivalent to an intruder test) in order to equate their experience with males receiving an intruder test. Subjects in groups 1 and 2 were videotaped when they were housed with a female for 24 h, and the tape was reviewed to verify mating in group 1 and the absence of mating in group 2. After the behavioral test, the intruder was removed and the subject was left in the home cage for 60 min without disturbance. Subjects were then anesthetized, perfused, and their brain sections were processed for Fos immunocytochemistry (see below).

In a second experiment, male prairie voles were mated with a sexually receptive female for 24 h while behavior was videotaped to verify mating. Females were removed and male subjects were housed alone for 24 h. Subjects were then divided into groups that received a 6-min intruder test by using either a sexually naive male ($n = 6$) or a female ($n = 5$), or a female partner ($n = 4$) as an intruder. A fourth group did not receive an intruder test (control, $n = 4$). Each subject's behavior was videotaped and scored. The intruder was removed at the end of the behavior test. One hour later, subjects were anesthetized, perfused, and their brain sections were processed for Fos immunocytochemistry.

2.3. Resident–intruder test

A socially reared sexually naive male prairie vole, that is usually not aggressive, was used as an intruder in the resident intruder test [45]. The intruder was placed in the home cage of the subject for 6 min while behavioral interaction between the subject and the intruder was recorded and scored by using a computerized data acquisition system. Mated males either offensively attacked an intruder by biting, pushing, threatening and chasing the intruder, or displayed defensive upright posture towards an intruder [45]. Frequency of subject's offensive and defensive aggression as well as durations of social activities

such as sniffing, huddling, and retreating over the 6-min observational period were recorded.

2.4. Fos immunocytochemistry

Sixty minutes after the behavioral test, all subjects were anesthetized using sodium pentobarbital (1 mg/10 g body weight), and perfused through the ascending aorta with 0.9% saline followed by 5% acrolein in 0.1 M phosphate buffer (pH 7.6). Brains were removed and 40-μm transverse sections were cut on a microtome. Floating sections at an 80-μm interval were processed for Fos immunocytochemistry. Brain sections were incubated in 20% goat serum in 0.05 M Tris-NaCl with 0.5% Triton (Tris-Triton) for 10 min; rabbit anti-Fos serum (Santa Cruz Biotech., CA) 1:1000 in Tris-Triton with 2% goat serum (Tritrigo) for 90 min at 37°C; biotinylated goat-anti-rabbit in Tritrigo for 90 min at room temperature; and avidin–biotin–peroxidase complex in Tris-NaCl for 60 min. Sections were stained by 0.05% 3,3′-diaminobenzidine in Tris-NaCl with 0.0015% H_2O_2 for 20 min. To reduce variability in the background, sections from all subjects were processed concurrently for Fos immunocytochemistry. Control sections were incubated with rabbit anti-Fos serum that was pretreated with 10 fold c-Fos control peptide (Santa Cruz Biotech.), which eliminated specific staining.

2.5. Data quantification and analysis

Diagrammatic representative brain sections from an atlas [41] and serial Nissl-stained brain sections from a prairie vole were used for locating and numbering of cells that contained Fos-like immunoreactivity. Fos-like immunoreactivity was measured by using computerized gray-level thresholding (IMAGE 1.57 developed by Wayne Rasband at NIH) and was counted in the lateral septum (LS); medial preoptic area (MPO); the bed nucleus of the stria terminalis (BST; anterodorsal and anteroventral areas); and the amygdala (AMYG; medial (me), cortical (co) and basolateral (bl) areas). Fos-ir cells were also counted in the piriform cortex, which was selected as a control region because exposure to a pup and exhibiting parental behavior did not induce Fos-ir staining in this area in prairie voles

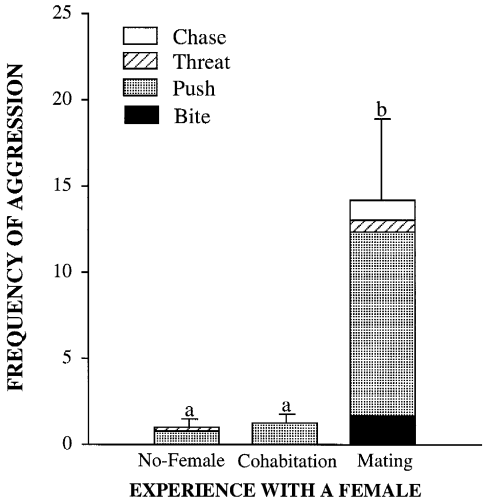

Fig. 1. Effects of prior experience with a female on resident–intruder aggression in male prairie voles. Males that mated with a female (Mating) exhibited more aggression towards a male intruder than males that cohabited with a female without mating (Cohabitation) or that had no prior experience with a female (No-female). The alphabetic letters illustrate group differences based on post hoc test (SNK) following ANOVA ($P < 0.05$). Error bars indicate standard errors of the means for composite score of aggression.

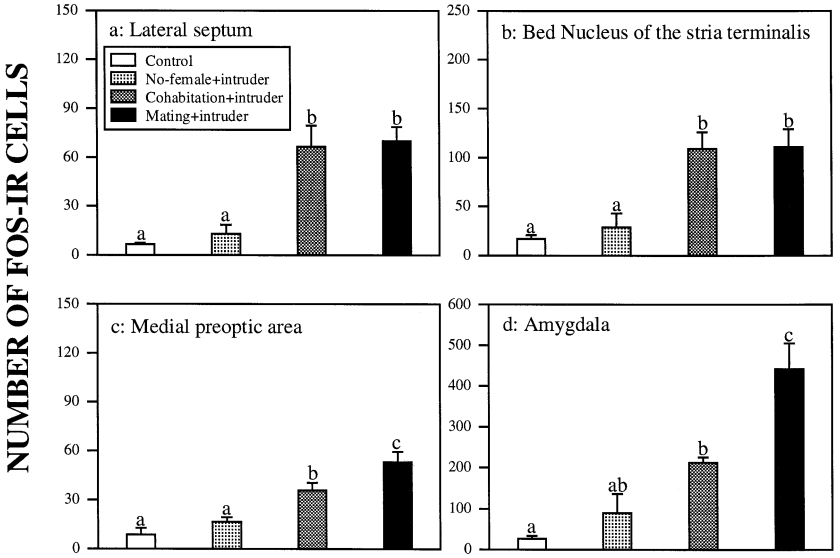

Fig. 2. Mean number of Fos-ir cells in the lateral septum (a), bed nucleus of the stria terminalis (b), medial preoptic area (c) and amygdala (d) in male prairie voles that had neither prior experience with a female nor intruder test (control), had no female experience and received an intruder test, cohabited with a female without mating and received an intruder test, or mated with a female and received an intruder test. The alphabetic letters illustrate group differences based on post hoc test (SNK) following ANOVA ($P < 0.05$). Error bars indicate S.E.M.

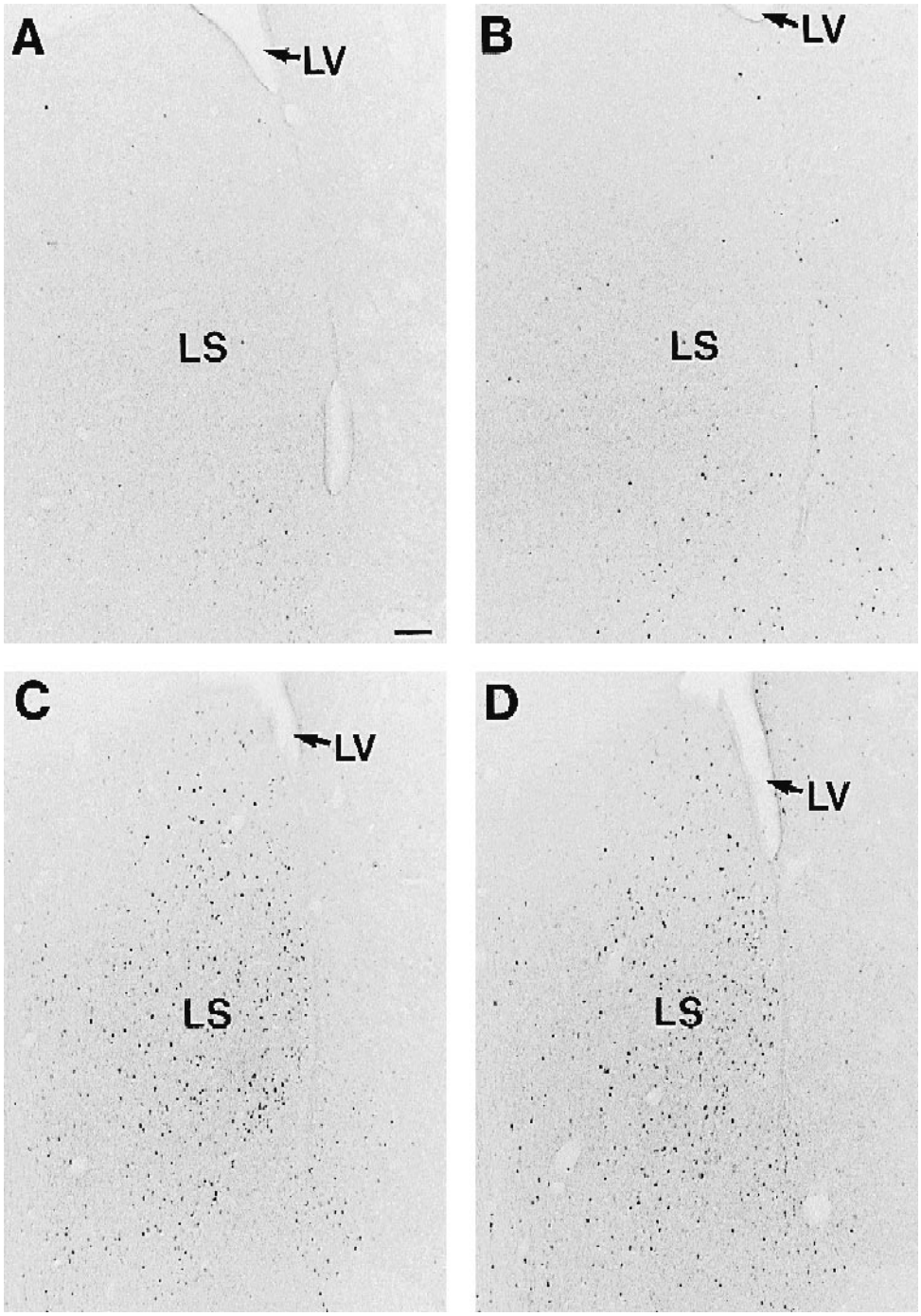

Fig. 3. Photomicrographs displaying Fos-ir cells in the lateral septum (LS) of representative male prairie voles that had neither prior experience with a female nor intruder test (A), had no female experience and received an intruder test (B), cohabited with a female without mating and received an intruder test (C), or mated with a female and received an intruder test (D). LV, lateral ventricle. Scale bar = 100 μm.

[29]. The number of pixels representing images of Fos-like immunoreactivity was determined in each of the above-mentioned brain areas, and was counted bilaterally. The number of pixels was converted into number of cells containing Fos-like immunoreactivity using a linear regression curve. The regression was obtained by correlating the

Fig. 4. Photomicrographs displaying Fos-ir cells in the amygdala (AMYG) of representative male prairie voles that had neither prior experience with a female nor intruder test (A), had no female experience and received an intruder test (B), cohabited with a female without mating and received an intruder test (C), or mated with a female and received an intruder test (D). OT, optic tract. Scale bar = 100 μm.

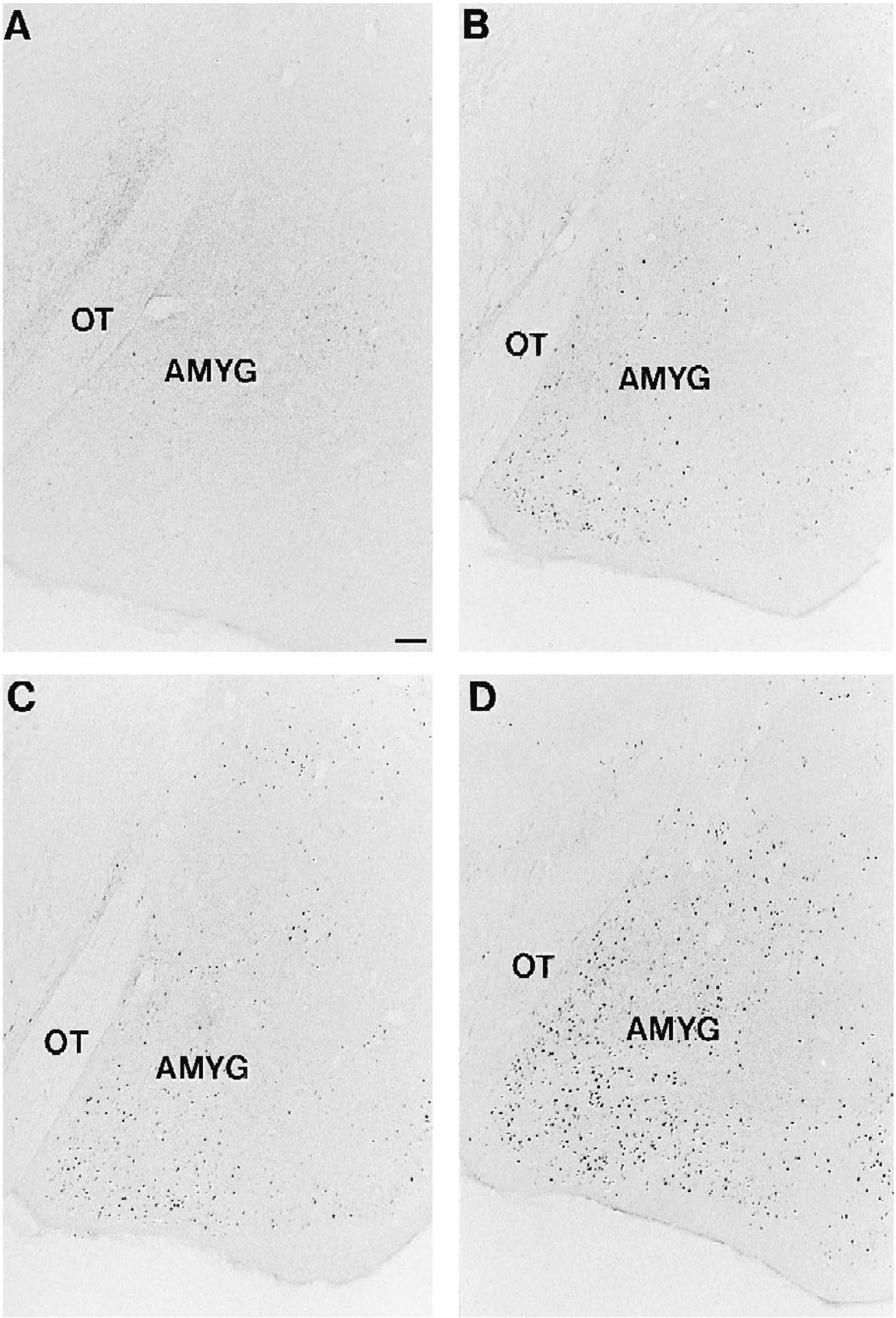
A
OT
AMYG
B
OT
AMYG
C
OT
AMYG
D
OT
AMYG

Table 1
Effects of the female experience on the number of Fos-ir cells in the amygdala

Group	Treatment	Amygdala		
		Medial	Cortical	Basolateral
I	No-female and no intruder	7.5 ± 1.4	8.2 ± 2.0	10.8 ± 4.7
II	No-female and intruder	31.5 ± 16.5	32.5 ± 16.8	26.0 ± 14.0
III	Cohabitation and intruder	61.3 ± 3.8	103.2 ± 17.7	48.0 ± 3.8
IV	Mating and intruder	148.7 ± 24.4	196.0 ± 34.5	97.4 ± 11.5
	$P <$	0.001	0.001	0.001
	SNK test	I,II,III < IV	I,II < III < IV	I,II,III < IV; I < III

number of pixels with the number of Fos-ir cells that were counted individually in 30 sampling areas with counts of Fos-ir cells ranging from 3 to 673 (correlation coefficient $r = 0.99$). Slides were coded so that the experimenter was blind to the identity of the specimens. The average number of cells containing Fos-ir from both sides of each brain

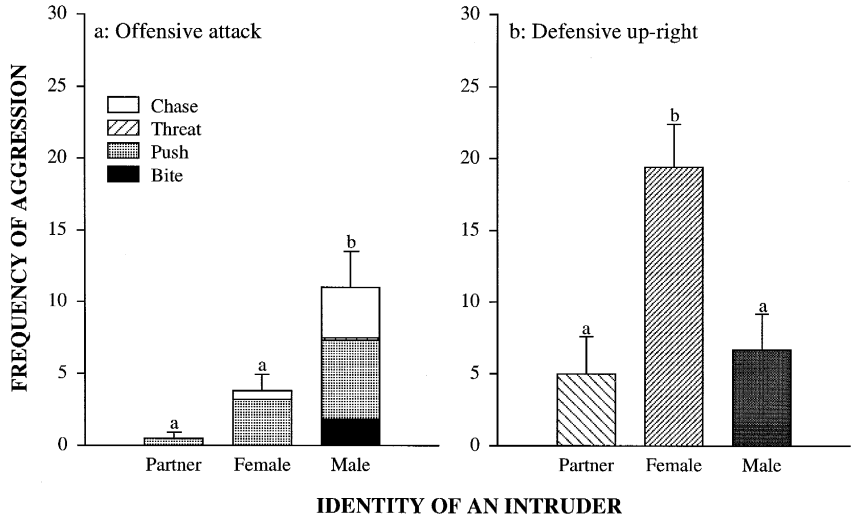

Fig. 5. Mating-induced selective aggression to different intruders. Male prairie voles showed more offensive aggression towards a male intruder than towards a female intruder or a female partner (a), and more defensive upright posture towards a female intruder than towards a male intruder or a female partner (b). The alphabetic letters illustrate group differences based on post hoc test (SNK) following ANOVA ($P < 0.05$). Error bars indicate S.E.M.

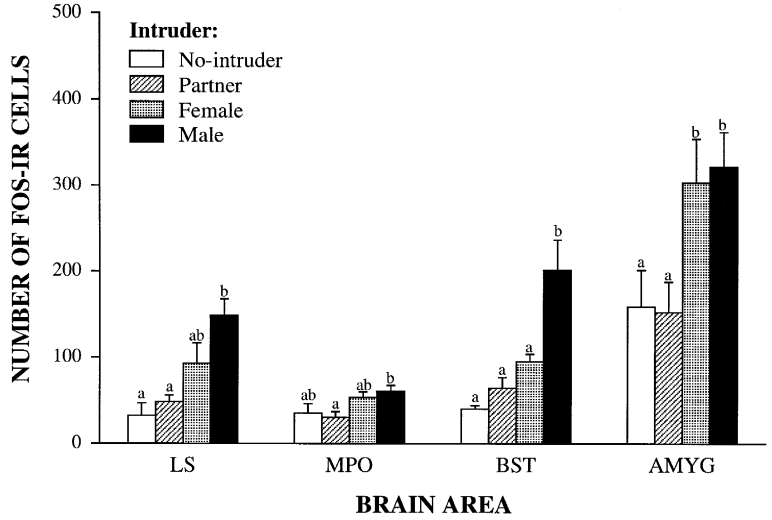

Fig. 6. Mean number of Fos-ir cells in the lateral septum (LS), medial preoptic area (MPO), bed nucleus of the stria terminalis (BST) and the amygdala (AMYG) in mated male prairie voles that did not receive an intruder test, or received an aggression test towards a female partner, a female intruder, or a male intruder. The alphabetic letters illustrate group differences based on post hoc test (SNK) following ANOVA ($P < 0.05$). Error bars indicate S.E.M.

area was used to provide individual means for data analysis. The frequency and duration of behaviors and number of Fos-ir cells in each brain area were analyzed by using a one-way analysis of variance (ANOVA) for each experiment. Significant treatment effects were further evaluated using a Newman–Keul's post hoc test (SNK).

3. Results

3.1. Effects of prior experience with a female on selective aggression

Differential experience with a female significantly altered the male's behavior to an intruder. Males that mated

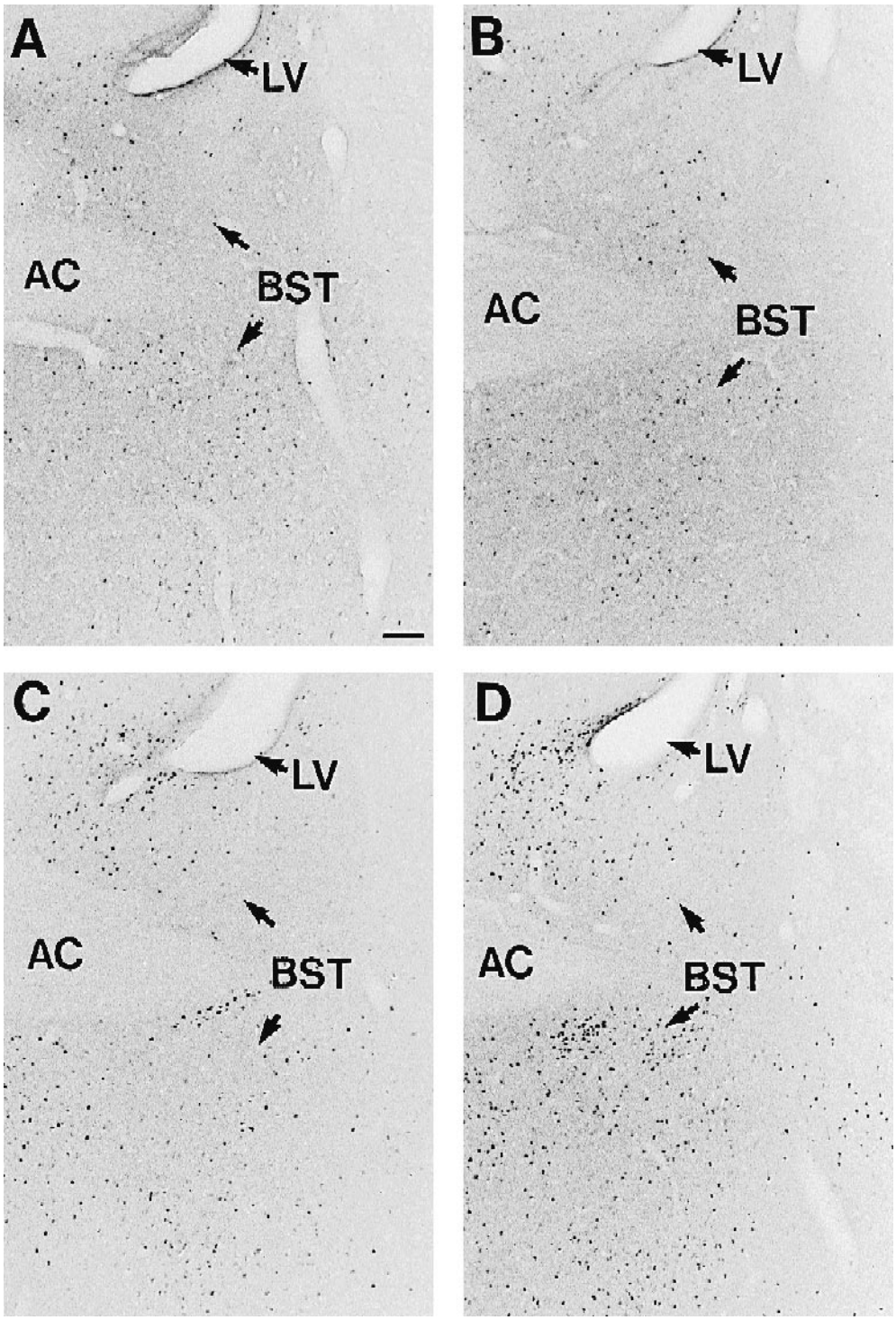

Fig. 7. Photomicrographs displaying Fos-ir cells in the bed nucleus of the stria terminalis (BST) of mated male prairie voles that did not receive an intruder test (A), or that received an aggression test towards a female partner (B), a female intruder (C), or a male intruder (D). AC, anterior commissure. Scale bar = 100 μm.

with a female for 24 h showed more offensive aggression to a male intruder compared to males that cohabited with a female without mating or that had no prior experience with a female ($F = 4.87$, df $= 2/12$, $P < 0.05$; Fig. 1). Mated males bit the intruder more than males in other experimental groups ($F = 4.50$, df $= 2/12$, $P < 0.05$). No treatment effect was found either in the defensive upright posture or in other social activities such as sniffing, huddling and retreating.

3.2. Interaction of prior female experience and an intruder test on Fos-ir staining

Differential experience with a female was also associated with different patterns of regional Fos-ir staining induced by an intruder test. Males that had prior experience with a female (mating or cohabitation) had more Fos-ir cells in the LS ($F = 21.30$, df $= 3/17$, $P < 0.01$; Fig. 2a and Fig. 3) and BST ($F = 13.35$, df $= 3/17$, $P < 0.01$; Fig. 2b) than males without female experience. Mated males had more Fos-ir cells in the MPO ($F = 18.28$, df $= 3/17$, $P < 0.01$; Fig. 2c) and in the amygdala ($F = 20.45$, df $= 3/17$, $P < 0.01$; Fig. 2d and Fig. 4) than any other males. Cohabited males, in turn, had more Fos-ir cells either in the MPO than males without female experience, or in the amygdala than males that had neither female experience nor intruder test. Analysis of Fos-ir cells in each subdivision of the amygdala Table 1) indicated that mated males had more Fos-ir cells in the medial (AMYGme), cortical (AMYGco) and basolateral subnuclei (AMYGbl) than any other males. Cohabited males had more Fos-ir cells in the AMYGco than males without female experience, or in the AMYGbl than males with neither female experience nor intruder test. Finally, mated males had more Fos-ir cells in the piriform cortex than males that had neither female exposure nor intruder test ($F = 4.23$, df $= 3/17$, $P < 0.05$).

3.3. Effects of the intruder's identity on selective aggression

Male prairie voles that mated with a female for 24 h behaved differently with the three kinds of intruders. They exhibited more offensive aggression to a male intruder than to a female intruder or a female partner ($F = 6.75$, df $= 2/12$, $P < 0.05$; Fig. 5a). They also showed more defensive aggression (upright posture) to a female intruder than to a male intruder or a female partner ($F = 7.43$, df $= 2/12$, $P < 0.01$; Fig. 5b). The experimental groups, however, did not differ in other social activities such as sniffing, huddling and retreating.

3.4. Fos-ir induction associated with different intruders

Exposure to different intruders also was associated with unique patterns of regional Fos-ir staining (Fig. 6). Males exposed to a male intruder had more Fos-ir cells in the MPO than males exposed to a female partner ($F = 3.81$, df $= 3/15$, $P < 0.05$) or in the LS than males exposed to a female partner or males that had no intruder test ($F = 7.81$, df $= 3/15$, $P < 0.01$). In addition, males exposed to a male intruder had more Fos-ir cells in the BST than any other males ($F = 9.68$, df $= 3/15$, $P < 0.01$; Fig. 7). In the amygdala, males exposed to a female intruder had similar number of Fos-ir cells as the males exposed to a male intruder, which was significantly more than males exposed to a female partner or had no intruder test ($F = 4.24$, df $= 3/15$, $P < 0.05$). A detailed analysis on number of Fos-ir cells in the subdivisions of the amygdala is summarized in Table 2. Males exposed to a male or a female intruder had more Fos-ir cells in the AMYGme than males exposed to the female partner or males that had no intruder test, or in the AMYGbl than the group that had no intruder test. Finally, the number of Fos-ir cells in the piriform cortex was not different among treatment groups.

Table 2
Effects of different intruders on the number of Fos-ir cells in the amygdala

Group	Intruder	Amygdala		
		Medial	Cortical	Basolateral
I	None	60.7 ± 14.8	75.4 ± 24.8	40.2 ± 7.9
II	Partner	44.7 ± 8.3	72.9 ± 24.8	61.0 ± 8.8
III	Female	111.1 ± 21.0	146.1 ± 29.3	83.8 ± 11.3
IV	Male	118.1 ± 12.1	122.0 ± 21.4	85.9 ± 6.7
	$P <$	0.001	Not significant	0.001
	SNK test	I,II < III,IV	–	I < III,IV

4. Discussion

In male prairie voles, the emergence of selective aggression as a consequence of mating appears to represent a form of mate guarding [9] and an important component of pair bond formation [45]. Our data from the current study not only suggested the influence of the reproductive history and the intruder's identity on male aggression, but also indicated a pattern of neural activation associated with prior experience with a female or with mating-induced aggression in male prairie voles.

4.1. Mating-induced selective aggression

Prior experience with a female conferred significant differences on male prairie vole's behavior in a resident–intruder paradigm. Males that mated with a female for 24 h displayed more aggression towards conspecific intruders than males that cohabited with a female without mating or that had no prior experience with a female. The latter two groups, in turn, did not differ from each other. These data

confirmed earlier results demonstrating that the induction of male aggression during 24 h of cohabitation with a female is dependent upon mating [25,45]. As mated and cohabited males did not differ on other measures of social behavior during the 24 h cohabitation [25], differences in aggression could not be attributed to any aspect of the cohabitation experience other than mating. In addition, differences in aggression could not be attributed to differences in the opportunity to explore intruders because subjects in all treatment groups equally sniffed intruders.

Our data extended earlier results by demonstrating different patterns of aggression towards different intruders in prairie voles. Males displayed more offensive aggression to a male intruder but more defensive upright posture to a female intruder (Fig. 5). The contrast in the response towards a male versus a female intruder might reflect differences in the intruder's behavior. Sexually naive female intruders appeared more aggressive than sexually naive male intruders, initiating contact with the male resident or even offensively attacking the male. As a consequence, male residents frequently displayed the defensive upright posture in the presence of female intruders, but not in the presence of male intruders who were more submissive and rarely attacked residents. As expected, mated males did not exhibit aggression when their mates were re-introduced, indicating that prairie voles were able to recognize their mates after 24 h of separation. These findings are consistent with an earlier report that the male's aggression is selective and resembles territory or mate guarding [45] as observed in other monogamous species [9,31].

The mechanisms underlying mating-induced aggression are still unknown in prairie voles. In other rodents, olfactory stimulation from a female partner facilitates the male's aggression and reduces tolerance of unfamiliar conspecifics [6,15,22]. Testosterone has been suggested as an important intermediate [2]. Chemosensory cues have been implicated in the development of social behavior in prairie voles [30,44]. Mating increases plasma testosterone in males [43], and increased testosterone has been associated with increased aggression in prairie and other species of voles [19,21].

4.2. Regional Fos-ir induction

In the present study, Fos immunoreactivity was used as a marker of neural activation. Our data suggest that exposure to an intruder induces regional Fos-ir staining in male prairie voles that have had prior experience with a female. The pattern of neural activation, however, varies in males that have had different experience with a female or that have been exposed to different intruders.

Aggression towards a male intruder was associated with a massive increase in the number of Fos-ir cells in the amygdala, whereas exposure to an intruder without displaying aggression (cohabitation group) resulted in less Fos-ir staining in this area (Fig. 2). As both groups of males received equivalent olfactory cues (i.e. male intruder) and showed equivalent amounts of olfactory investigation, the increased Fos-ir staining in the amygdala in aggressive males may be a residual of prior sexual activity, may result from differential sensory processing of stimuli associated with the intruder, and/or may subserve the aggressive response. Results from the second study (Fig. 6), in which all males had mated but only those aggressive to a male or a female intruder had increased Fos-ir staining in the amygdala, exclude the possibility that these regional changes were a residual of prior sexual activity.

Our data indicated regional differences across subdivisions of the amygdala. In the AMYGco, for example, the number of Fos-ir cells was significantly increased after males had experience with a female (mating or cohabitation), but not after they were exposed to an intruder (Table 1). These data indicated that the AMYGco was probably aroused by social stimuli from the female [33], but did not respond to intruder-related cues. In the AMYGme, mated males that were aggressive to a male or a female intruder had more Fos-ir cells than either cohabited males (Expt. 1) or mated males that were not aggressive to a female partner (Expt. 2), suggesting that activation of this brain area was specifically associated with mating and its induced selective aggression. Mating increased Fos-ir staining in the AMYGme in several species of rodents including hamsters [32,33], rats [5] and gerbils [23]. In rodents, the AMYGme receives inputs from the accessory olfactory bulb [12,13,38] and, in turn, projects to the MPO, BST and LS [27]. These multisynaptic pathways are known to be involved in the regulation of male–male aggression [3,7,15], and in the control of reproductive events and processing of socially relevant cues [14,17,32,34,37]. In prairie voles, exposure to conspecific pups while displaying parental behavior increased Fos-ir staining in the AMYGme [29,42] similar to that observed in the present study and lesions of this area decreased paternal and affiliative behaviors [28], again suggesting an involvement of this structure in processing social cues and in regulation of motor responses. It is not possible in the present study, however, to determine whether increased Fos-ir staining was related to the perception of the stimuli or the aggressive response to an intruder.

Regional Fos induction by an intruder was not limited to mated males demonstrating aggression. Although cohabitation with a female without mating did not induce aggression, there was an increase in regional Fos-ir staining in this group. These males, along with mated males that were aggressive, had more Fos-ir cells in the BST and LS than males that had no prior experience with a female but also exposed to an intruder (Fig. 2). These data indicated that neither mating nor aggression was necessary for the elevation in Fos-ir staining in these areas, and apparently prior experience with a female induced changes in the responsiveness of these brain areas to social cues. This notion

was supported by previous studies. In the gerbil, Fos-ir expression in the BST and LS was associated with the male entering the sex arena, but no further increase was seen with mating [23]. In the hamster, an elevation of Fos-ir staining in the BST induced by mating was similar to that induced by exposure to female vaginal secretions [16] or by displaying agonistic behavior [33]. Projections from the BST to the LS have been implicated in the behavioral response to social stimuli in rats [35] and voles [29,42]. It may be that the experience with a female induced hormonal changes, which in turn act on receptive areas and modulate their sensitivity and responsiveness to external cues [18]. Although current data suggest an involvement of these brain areas in processing intruder-related cues, we cannot exclude the possibility that these areas were also implicated in behavior. In fact, that mated males exposed to a male intruder had more Fos-ir cells in the BST than the males exposed to a female intruder or a female partner indicated activation of this brain area associated with offensive male attack.

In the MPO, mated males had more Fos-ir cells than any other males (Fig. 2). Because the Fos-ir expression did not increase after males were exposed to a male or a female intruder (Fig. 6), differences between mated and other males are better to be explained by a residual of mating rather than the responses to intruders. That mating increases Fos-ir expression in the MPO has been reported in a variety of rodent species such as rats [5], mice [4], hamsters [16,33] and gerbils [23]. Although males that cohabited with a female without mating had less Fos-ir cells in the MPO than mated males, they had more Fos-ir cells than males that had no prior experience with a female (Fig. 2). These data indicate gradations of neural activation dependent on experience with a female. This two-step pattern of Fos-ir elevation was found in other rodents. In the gerbil, for example, Fos-ir expression in the medial sexually dimorphic area was increased when males interacted with a female, and then was further increased at ejaculation [23]. It is suggested that such increased Fos-ir expression in the absence of mating is a conditioned response that develops when social stimuli from the female are paired with genital somatosensory stimulation [23]. The physiological function of this pattern of neural activation is still unknown.

Activation of Fos-ir staining in some brain regions depended on the type of intruder-related cues or the behavioral pattern towards an intruder. The AMYGme was activated equally following exposure to male or female intruders but not to the female partner, suggesting an involvement of this area in response to stranger-related cues and in displaying offensive as well as defensive aggression. In the BST, however, Fos-ir expression was increased only when mated males were exposed to a male intruder but not to a female intruder or the female partner, indicating that this area was involved solely in processing male-related cues or in offensive aggression. These data may provide evidence to support the theory that offensive and defensive aggression represent distinct systems organized by discrete neural circuits [1,36]. Alternately, this difference might simply reflect differential processing of unique social cues independent of expression of aggressive behavior.

In the current study, presentation of male intruders had no effect on the number of Fos-ir cells detected in selected brain areas of resident voles that did not have prior experience with a female, suggesting that a 6-min exposure to an intruder may have been insufficient to induce a detectable changes in Fos-ir activity in those males. In addition, mated males that were re-exposed to their female partners had a similar amount of regional Fos-ir staining as males that did not receive an intruder test, indicating that these brain areas, measured by Fos-ir, were not activated by the partner-related stimuli. In the piriform cortex, there were more Fos-ir cells in mated males than in males without female experience (Expt. 1). In this region, males with or without an intruder test did not differ (Expt. 2). These data indicate that, as measured by Fos-ir, piriform cortex may be activated by sexual activity as reported in male rats [37], but not social stimuli per se. In an earlier study, Fos-ir staining in the piriform cortex was not increased by pup exposure in male prairie voles [29].

A guiding assumption in this study was that mating confers long-term changes on behavior and neural activity, and the latter can be detected by increased Fos immunoreactivity in an anatomically discrete circuit. The results from the current study suggest different regional neural activation depending on prior experience with a female or exposure to an intruder. Activation of the AMYGme was dependent on mating and linked to aggression. In this brain area, mating appears to produce a long-term change in responsiveness with profound behavioral results. Aggression against a male or a female intruder, therefore, was an essential condition for the induction of Fos-ir staining in the AMYGme. In contrast, activation of the BST (and LS) was dependent upon social (not sexual) experience with a female and appeared to be involved in the processing of male-related cues as well as in male–male aggression. Although the MPO was activated by social or sexual experience with a female, it did not respond to intruder-related stimuli, as measured by Fos-ir staining. Distinct neural systems involving the expression of behavior or processing olfactory cues were also found in a recent study of maternal behavior in mice [8].

It should be pointed out that results from Fos-ir staining are not conclusive. Neurons involved in processing intruder-related cues or in the regulation of aggression may express other immediate–early gene products that were not examined in the current study [24]. In addition, although increased Fos-ir staining may indicate regional neuronal activity, the physiological significance (i.e. activation vs. inhibition) of Fos-ir induction remains to be determined with other techniques.

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