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Olfactory Contribution to Fos Expression during Mating in Inexperienced Male Hamsters

Gwen D. Fernandez-Fewell[1] and Michael Meredith

Program in Neuroscience, Department of Biological Science, Florida State University, Tallahassee, FL 32306, USA

[1]Present address: Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229, USA

Correspondence to be sent to: Dr Michael Meredith, Department of Biological Science, Florida State University, Tallahassee, FL 32306-4340, USA. e-mail: mmered@neuro.fsu.edu

Abstract

Male hamsters are very dependent on chemosensory cues for normal mating behavior. We have previously reported that central vomeronasal pathways are intensely and selectively activated during mating or pheromonal stimulation. The contribution of main olfactory sensory input to the patterns of *c-fos* activation was investigated in this study. Sexually inexperienced male hamsters were either made anosmic by intranasal infusion of zinc sulfate or remained intact. Fos protein immunoreactivity was analyzed in main olfactory and vomeronasal pathways of the zinc sulfate-treated, anosmic animals after mating with receptive females for 45 min, and compared with Fos patterns seen in intact mating animals, some of which have been described in a previous publication. The zinc sulfate-treated anosmic males described here all mated when given access to receptive females. Whether mated or unstimulated, anosmic males had little or no Fos expression in main olfactory pathways; significantly less even than in unstimulated intact animals. Mating did not increase Fos expression in main olfactory pathways of intact animals over that of unstimulated intact controls. However, Fos expression in central vomeronasal pathways was significantly higher in mating anosmic males, as in intact males, compared with appropriate non-mating controls. Fos expression was significantly different between intact and zinc sulfate-treated anosmic mating males in only one area studied. The rostral anterior medial amygdala, known to receive a small olfactory terminal field, had significantly lower Fos expression in zinc sulfate-treated anosmic males that mated when compared with intact-mating animals. Thus, functional main olfactory input to the rostral vomeronasal amygdala can be demonstrated but does not appear to be critical for mating behavior in previously inexperienced male hamsters with intact vomeronasal organs. Other main olfactory input appears to have a negligible contribution to Fos-patterns in such animals.

Introduction

Chemosensory information through either the olfactory system or the vomeronasal organ (VNO) is essential for male hamster mating behavior. Removal of the olfactory bulbs, and combined lesions of the olfactory and vomeronasal receptor systems or of their central pathways, eliminates mating (Murphy and Schneider, 1970; Powers and Winans, 1973,1975; Meredith, 1980, 1986; Lehman and Winans, 1982). Removal of the vomeronasal organs (VNX) from sexually inexperienced male hamsters results in severe impairments in mating, especially on first exposure to a receptive female (Meredith, 1986; Meredith and Howard, 1991; Fernandez-Fewell and Meredith, 1995). Experienced males are less affected or unaffected by VNX, suggesting that they use olfactory input to sustain mating (Powers and Winans, 1975; Meredith, 1980, 1986; Johnston and Rasmussen, 1984; Pfeiffer and Johnston, 1994). However, males with lesions of main olfactory receptors (OLFX) mate normally, whether experienced or inexperienced (Powers and Winans, 1973; Winans and Powers, 1977; Meredith, 1980). In previous experiments we used immunocytochemical detection of Fos, the protein product of the *c-fos* gene, as an indicator of regional neural activation in inexperienced male hamsters mating with receptive females, or exposed to hamster vaginal fluid (HVF), a source of female pheromones (Fernandez-Fewell and Meredith,, 1994a). Most of the increased Fos protein expression attributable to mating or pheromone stimulation appeared in the vomeronasal pathways and their central targets, not in the main olfactory pathways. Here, we study the main olfactory contributions to this CNS activation during mating—by comparing activation in OLFX and intact males.

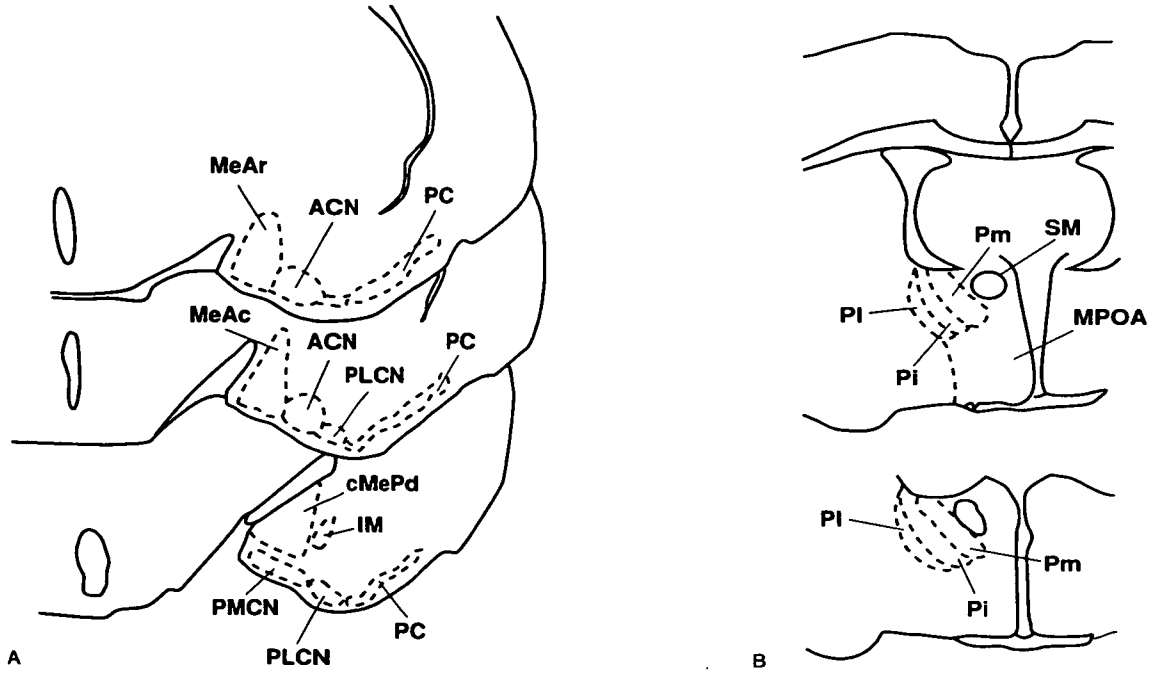

Figure 1 Microprojector drawings of boundaries of CNS regions within which Fos-positive nuclei were counted (in this and previous studies). **(A)** Coronal sections through the amygdala from rostral to caudal levels showing the corticomedial (MeA, MeP, PMCN) and corticolateral (ACN and PLCN) amygdaloid nuclei. MeAr, MeAc = rostral and caudal parts of anterior medial nucleus. cMePd = caudal part of dorsal posterior medial nucleus. IM = intercalated mass. Coronal sections through the medial preoptic area (MPOA) and posteromedial (Pm) division of the bed nucleus of the stria terminalis (BNST), both rostral (upper) and caudal (lower). SM = Stria medularis; PI, Pi = posterolateral and postero-intermediate divisions of BNST.

In our previous study, we found increased Fos expression that was dependent on vomeronasal (VN) sensory input, in the accessory olfactory bulb (AOB) and areas it projects to: the posteromedial cortical nucleus of the amygdala (PMCN) and the medial nucleus (Me). Activation in both anterior (MeA) and posterior (MeP) medial amygdala was partially dependent on an intact vomeronasal system, in that Fos expression was significantly lower in the few males that mated after VNX than in intact-mating males. However, activation in Me was also partially dependent on mating performance (copulation), in that Fos expression was significantly higher in VNX-mating males than in (the majority of) VNX males that failed to mate. In the medial preoptic area (MPOA) and the rostral posteromedial bed nucleus of the stria terminalis (BNST)—regions which receive input from Me and are known to be important for mating behavior (Powers *et al.*, 1987)—Fos expression in previously inexperienced males was dependent on mating but not on VN input. Fos expression in the caudal BNST was correlated with chemoinvestigatory behaviors (Fernandez-Fewell and Meredith, 1994a) but not dependent on an intact VN system or on copulatory performance. Increased expression in all these areas during mating or chemosignal exposure in intact animals has also been reported by others for both hamsters (Kollack and Newman, 1992; Fiber *et al.*, 1993; Kollack-Walker and Newman, 1997) and rats (Baum and Everitt, 1992; Wersinger *et al.*, 1993; Coolen *et al.*, 1997). The location of these CNS areas are shown in Figure 1.

In contrast to these VN projection areas, Fos expression in the main olfactory pathways in mating, inexperienced males was not obviously different than that in males placed in clean cages without females (unstimulated controls) (Fernandez-Fewell and Meredith, 1994a), although some Fos expression was always present in olfactory areas in both groups. Detailed data on Fos expression in main olfactory pathways were not included in that previous report, nor was there an assessment of the main olfactory contribution to Fos patterns in the VN pathways. This question is addressed here, using relevant data from previous experiments.

The present experiments were designed to demonstrate any main olfactory contribution to Fos patterns elicited by mating in sexually inexperienced animals, by studying animals with main olfactory lesions that spared VN sensory input. We know from our own work, and that of others, that anosmic animals mate normally. Thus, Fos expression due solely to VN sensory input or activation related to mating performance would be normal, but any significant loss of Fos expression due to loss of main olfactory input could be analyzed. The main olfactory input, via the main olfactory bulb, distributes widely in the brain to the anterior olfactory nucleus (AON), olfactory tubercle (OT), pyriform cortex (PC) and entorhinal cortex (EC) (Scalia and Winans, 1975; Davis *et al.*, 1978; Kevetter and Winans, 1981b). It also

projects to the corticolateral or 'olfactory' amygdala, which includes the anterior cortical (ACN) and posterior lateral cortical (PLCN) nuclei (Kevetter and Winans, 1981b). Intra-amygdaloid connections connect the 'olfactory' amygdala with the 'vomeronasal' amygdala (MeA, MeP and PMCN) which receives VN input via the AOB (Barber and Raisman, 1974; Scalia and Winans, 1975; Davis *et al.*, 1978; Kevetter and Winans, 1981a,b; Shammah-Lagnado and Negrao, 1981). There is also a small, direct, olfactory terminal-field in the most rostral part of MeA (Lehman and Winans, 1982; Gomez and Newman, 1992). These cross-connections may be important for integration of chemosensory information during mating. Fos patterns within the Me, previously attributed to VN sensory input, may be influenced by direct or indirect contributions from main olfactory projections to Me. Thus, selectively eliminating main olfactory receptors allows us to look for any main olfactory contribution to previously described Fos patterns within the amygdala and elsewhere, especially those regions of the VN pathway where increased Fos activation has been attributed to mating or to chemosensory input in intact animals.

In this study, main olfactory lesions were made with 0.17 M zinc sulfate in ether-anesthetized, sexually inexperienced, male hamsters 3 days before they were tested for mating behavior. After behavioral stimulation and mating, Fos expression in central olfactory and VN pathways was compared between previously inexperienced, intact and OLFX animals, both mating and unstimulated. The intact animals were part of a previous experiment (Fernandez-Fewell and Meredith, 1994a) but the data on Fos expression in their main olfactory pathways is reported here for the first time. The treatment of mating and unstimulated animals and the details of Fos immunocytochemistry and of the counting of nuclei were identical in the two experiments.

Materials and methods

Zinc sulfate lesions

Main olfactory lesions were made by intranasal infusion of zinc sulfate (Alberts and Galef, 1971; Alberts, 1974), a non-surgical procedure shown to spare VN input (Mayer and Rosenblatt, 1993). After infusion with 0.17 M zinc sulfate, there appears to be no regeneration in areas where the receptor epithelium is sloughed off from the lamina propria (Winans and Powers, 1977; Harding *et al.*, 1978), although not all areas are equally damaged and there is usually some regeneration. Behavioral anosmia is brief in the hamster—only 8–10 days (Powers and Winans, 1973) or less (Meredith and O'Connell, 1988)—and the degree of protection of the VN receptors may depend on the method used. Ether anesthesia (Winans and Powers, 1977; Meredith, 1980) may protect the VN epithelium by increasing secretions in and around the VN duct (Meredith, 1980). Our method was modified from Mayer and Rosenblatt (1993). Zinc sulfate solution was sprayed into each nostril from a 1 ml syringe with a modified 20 ga hypodermic needle. The tip was blunted and pinched into a narrow slit, and the shaft bent so that liquid forced through the needle emerged in a narrow spray at ~45° to the shank. Sexually inexperienced male hamsters, 2–3 months old, were anesthetized with ether and placed prone on an inclined surface with the head down. The needle was inserted into each nostril in turn and the 0.1 ml of a 5% zinc sulfate solution (0.17 M) was forcibly expelled with the spray directed dorsally towards the olfactory area. Animals were held for 1 min in a head down position, while any solution draining from the nostrils was aspirated. This procedure appears less stressful to the animals, judged by recovery time and the behavior during recovery, than retro-nasal infusion of zinc sulfate solution via a tube inserted through the mouth and hooked around the soft palate (Powers and Winans, 1973). Animals were allowed to recover for 3 days before testing for anosmia and mating. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Florida State University.

Food finding tests

Animals were tested 3 days after zinc sulfate olfactory lesions (OLFX) for their ability to detect food odors (see Meredith and O'Connell, 1988). Briefly, the animals were food-deprived for 24 h and placed in a test chamber made of black Plexiglas except for a transparent front wall. The chamber had two ports in the floor 8 cm apart and 8 cm from the side and back walls. Under one port was a beaker of food pellets; under the other a beaker of corks of a similar size to the food pellets. The chamber was cleaned between tests and the side on which the food was placed was varied randomly. The time each animal spent sniffing and biting at each port was measured for a period of 3 min and a correct discrimination was recorded if the time spent at the food port was a minimum of 9 s and at least double that at the non-food port. If <2 s was recorded at either food port the test was repeated. Although this test for anosmia is a relatively crude test of olfactory capability, we know that rodents do not require much viable olfactory epithelium to be able to detect and discriminate odors (Slotnick and Guttman, 1977), so are confident that failure in the food test is a good measure of anosmia. Intact and VNX animals routinely pass this test on the first trial; the occasional failure is most often associated with infection in the nasal cavity. All OLFX animals included in the experiments reported here failed the food test on two separated trials. These animals spent approximately equal times at the food and cork ports. They include four OLFX animals that mated normally throughout the 45 min with receptive females, and three OLFX unstimulated controls. Three OLFX animals that did not mate throughout the 45 min exposure to females were dropped from the experiment.

Stimulation protocol

Intact and OLFX animals were placed in a clean plastic cage (40 × 20 × 19 cm) with fresh bedding and left for 1 min to habituate to their surroundings before being presented with a receptive female and allowed to mate for 45 min. The female was changed 1–2 times during this period for maximal stimulation. At 45 min the female was removed and the male left alone for another 45 min before being perfused for Fos immunocytochemistry. 'Unstimulated' control animals were placed in a clean cage with fresh bedding for 90 min and the cage was opened at least twice to provide some arousing stimulus during the initial 45 min period.

Immunocytochemical procedures

All animals were deeply anesthetized with Nembutal and perfused through the heart with 0.1 M phosphate buffered saline (pH 7.4) followed by 4% paraformaldehyde. The brains were removed and the olfactory bulbs were detached and embedded in gelatin (300 bloom). Both the brain and bulbs were postfixed overnight in 4% paraformaldehyde. The noses of OLFX animals were postfixed separately and later decalcified and sectioned for verification of the lesion (See Histological Analysis). Serial sections of the olfactory bulbs and of the rest of the brain were cut in horizontal and coronal planes, respectively, at 50 μm thickness on a Vibratome. Free-floating sections were washed in 0.1 M phosphate buffer and incubated with sheep Fos polyclonal antibody (Cambridge Research Biochemicals, OA-11-823 at a dilution of 1:40 000 or OA-11-824 at a dilution of 1:20 000) in 0.1 M PBS with 0.4% Triton X-100 for 48 h at 4°C. This affinity purified polyclonal antibody has been shown to detect Fos and Fos-related proteins. Therefore, in our results, the term 'Fos' can be considered to include both. After rinsing in 0.1 M phosphate buffer the sections were incubated with a biotinylated secondary antibody, rabbit anti-sheep IgG at 1:600 for 1 h at room temperature, rinsed in 0.1 M phosphate buffer and then incubated in an avidin–biotin–horseradish-peroxidase conjugate (ABC standard elite kit; Vector Labs) at a dilution of 1 drop A + 1 drop B/10 ml of 10 mM phosphate buffered saline for 1 h at room temperature. Sections were washed in 0.1 M phosphate buffer and stained with 3,3′-diaminobenzidine intensified with nickel. The sections were washed in 0.1 M phosphate buffer and then mounted on gelatin/chrome alum-coated glass slides, air dried, dehydrated through graded ethanol solutions, cleared with xylene and cover slipped with Permount. The presence of Fos was evident as a black reaction product in cell nuclei. Negative (no primary antibody) and LHRH-preabsorption controls for the specificity of staining with this system have been described previously (Fernandez-Fewell and Meredith, 1994a).

Counting protocol

Differences between the groups were quantified by counting all densely stained Fos-immunoreactive cell nuclei within equivalent sections in each experimental animal for each neuroanatomical nucleus of interest (1–3 sections per region per animal). In all animals equivalent sections for each region were selected on the basis of known neuroanatomical landmarks. The area counted was determined by the extent of the neuronatomical nucleus on the selected section. In the MOB, stained nuclei were counted throughout all parts of all layers of the MOB tissue on three sections, one at each of three horizontal levels, in each animal. The levels were (1) dorsal to the AOB, (2) at the level of the AOB and (3) ventral to the AOB. These counts were then averaged for each animal and divided by 5 so that the data for all main olfactory targets could be plotted on the same axes. The counts for the OT and PC were restricted to layer II and were made at three coronal levels, ~600 μm apart rostral to caudal. In the medial amygdala, counts of the MeA were made in two sections, one where the MeA first appears, and a second ~250 μm more caudal. Counts for the ACN were also made at these levels. Within the caudal medial amygdala, the count was made in the dorsal region (MeP). The ventral region at that level is the PMCN which was also analyzed. The Fos-positive nuclei in each region of interest were counted visually, at ×160 magnification, and the numbers averaged for each region across all animals in that group. Fos nuclei were not uniformly stained but densely stained nuclei (which were counted) could be distinguished from lightly stained nuclei with good reproducibility. All sections were counted by the same observer (GFF) and some were recounted to test reliability. The illustrations of Kollack and Newman (1992), along with a series of cresyl violet-stained hamster brain sections, were used as guidelines for nuclear boundaries. Outlines of the central regions counted are indicated in Figure 1. The graphs show the average numbers of Fos stained nuclei (± SEM) per neuroanatomical nucleus for each area of interest, not density per unit area.

Statistical analysis

The data from four mating and three control OLFX animals were analyzed along with data from eight mating and seven control intact animals obtained in a previous experiment (Fernandez-Fewell and Meredith, 1994a), as explained above. Differences in the number of Fos-positive nuclei within a region, between the four groups, were analyzed using a two-way analysis of variance (ANOVA; SigmaStat, Jandel Scientific Software) as a function of lesion (OLFX, intact) and stimulation (mating, unstimulated). Post-hoc multiple comparison tests used Student's Newman–Keuls tests. All P values considered significant were at $P < 0.05$.

Results

Because our object was to study Fos expression in anosmic animals that mated normally, OLFX animals that were able to locate food in the food-finding test or which appeared to be abnormal in their mating behavior were excluded from the experiment. All animals were treated similarly and mating was monitored during the 45 min exposure to females. All the OLFX animals actually failed in the food-finding test and those included in this study all appeared to mate normally when presented with a receptive female, although quantitative estimates of behavior were not recorded. Fos patterns in main olfactory pathways after mating are first described for OLFX mating (n = 4) and OLFX unstimulated animals (n = 3), and then compared with previously unreported data on Fos expression in the same areas of intact-mating (n = 8) and intact-unstimulated animals (n = 7), used in a previous experiment (Fernandez-Fewell and Meredith, 1994a). Other than the OLFX procedure, the intact animals were treated as described here for the OLFX animals and the Fos processing was identical (see Fernandez-Fewell and Meredith, 1994a). Fos expression in central VN pathways and the MPOA and BNST of OLFX animals are also described and compared with those previously described (Fernandez-Fewell and Meredith, 1994a) in the intact-mating and intact-unstimulated animals.

Histological analysis

Immediately after behavioral testing (3 days after zinc sulfate lesions), all OLFX experimental animals were perfused and their olfactory mucosae examined histologically. Figure 2A illustrates the appearance of normal olfactory epithelium in the posterior nasal cavity of an intact animal. In all seven OLFX animals the olfactory mucosa was necrotic and most of the epithelium was sloughed off (Figure 2B), or was in the process of sloughing off, from the underlying lamina propria. In many areas the underlying lamina propria appeared to be bare of any epithelial covering. In contrast, the VN epithelium of all OLFX animals appeared normal throughout its length. The ducts entering the organs were patent; the supporting cells and receptor cell layer did not show any signs of necrosis (Figure 2C).

Fos expression in main olfactory pathways of inexperienced OLFX- and intact-mating animals

OLFX lesions drastically reduced Fos expression in main olfactory pathways of OLFX males, mating or unstimulated, as expected (Figure 3 B,D,F). The main olfactory targets analyzed, i.e. the MOB, OT, PC, ACN and PLCN, showed low or no Fos expression in either OLFX-mating or -unstimulated animals (graph, Figure 4). Figure 1 shows outlines of the central neuroanatomical areas within which Fos expression was analyzed. In intact-mating animals

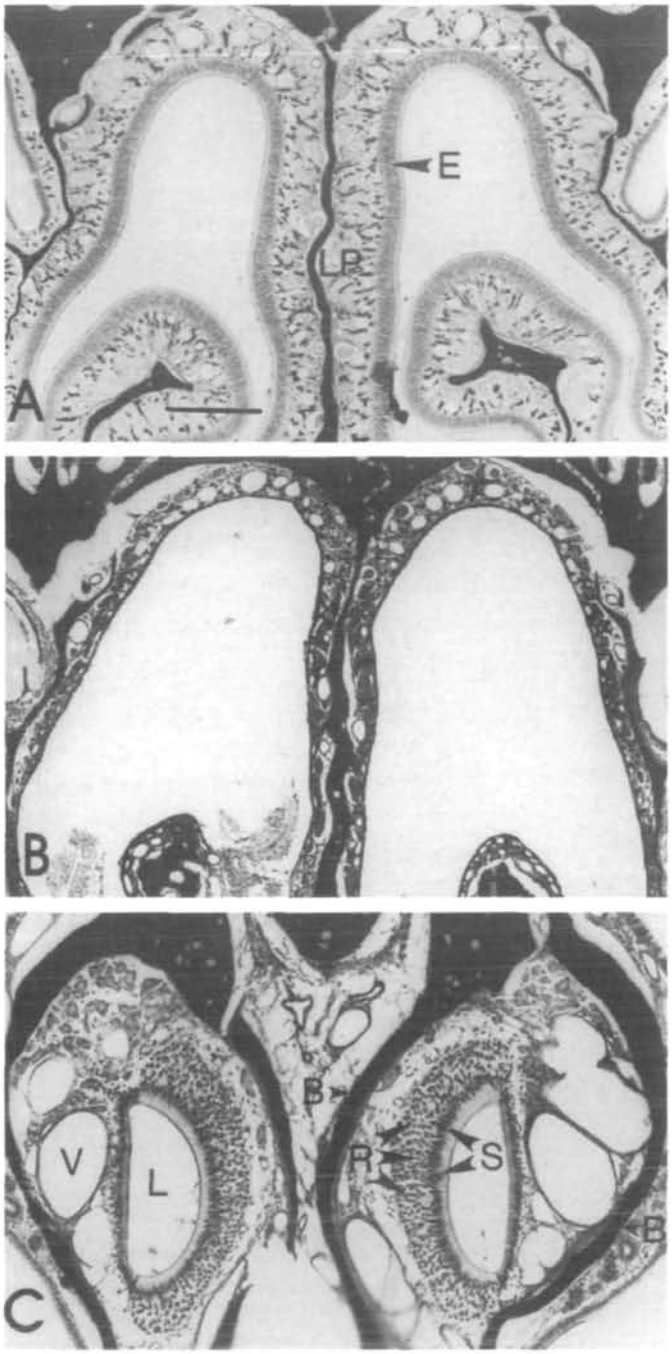

Figure 2 **(A–C)** Coronal sections through the nasal cavity of intact and OLFX animals showing olfactory and vomeronasal epithelium. (A) Normal olfactory epithelium in the posterior part of the nasal cavity in an intact animal. (B) Sloughed off olfactory epithelium in an OLFX animal, 3 days after treatment with zinc sulfate. (C) VNO of an OLFX animal showing no sign of damage. B = bony capsule of the VNO, E = olfactory epithelium, L= lumen, LP = lamina propria, V = vascular plexus, R = receptor cell layer, S = supporting cells. Scale bar in A refers to all sections as follows A, B, 350 μm; C, 420 μm.

(Figure 3A,C,E), Fos expression in the MOB, OT, PC and the olfactory amygdala (ACN and PLCN) was higher than that in OLFX animals, but not significantly different (except

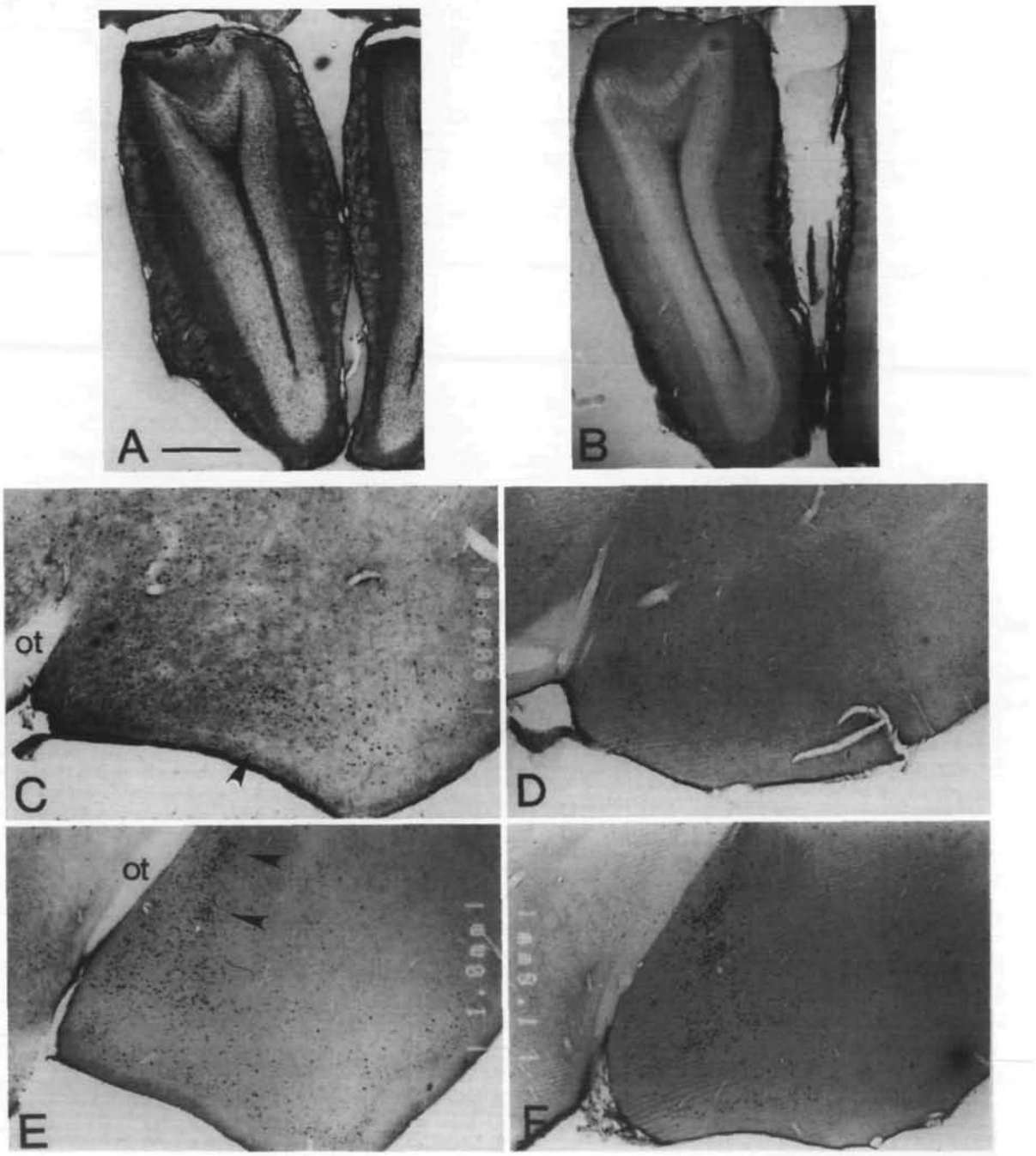

Figure 3 (A–F)

for PLCN) from that in intact unstimulated animals (Figure 4). However, the overall activation of the PLCN was very low even in mating animals (Figure 3F). No increase over the appropriate control level was seen in pheromonally stimulated animals (Fernandez-Fewell and Meredith, 1994b).

Two-way ANOVA analyses, comparing mating and unstimulated OLFX animals with mating and unstimulated intact animals showed a significant effect of OLFX-lesion on Fos expression in the MOB [$F(1,15) = 57.26$; $P < 0.0001$], the OT [$F(1,15) = 55.5$, $P < 0.0001$], the PC [$F(1,15) = 52.5$, $P < 0.0001$], the ACN [$F(1,15) = 51.32$, $P < 0.0001$] and the PLCN [$F(1,15) = 48.03$, $P < 0.0001$]. These areas had little or no Fos expression in OLFX animals, mating or unstimulated, and had equivalent levels of expression in intact animals whether mating or unstimulated, showing a significant effect of OLFX lesion but no effect of mating alone (with the exception of the PLCN of intact animals).

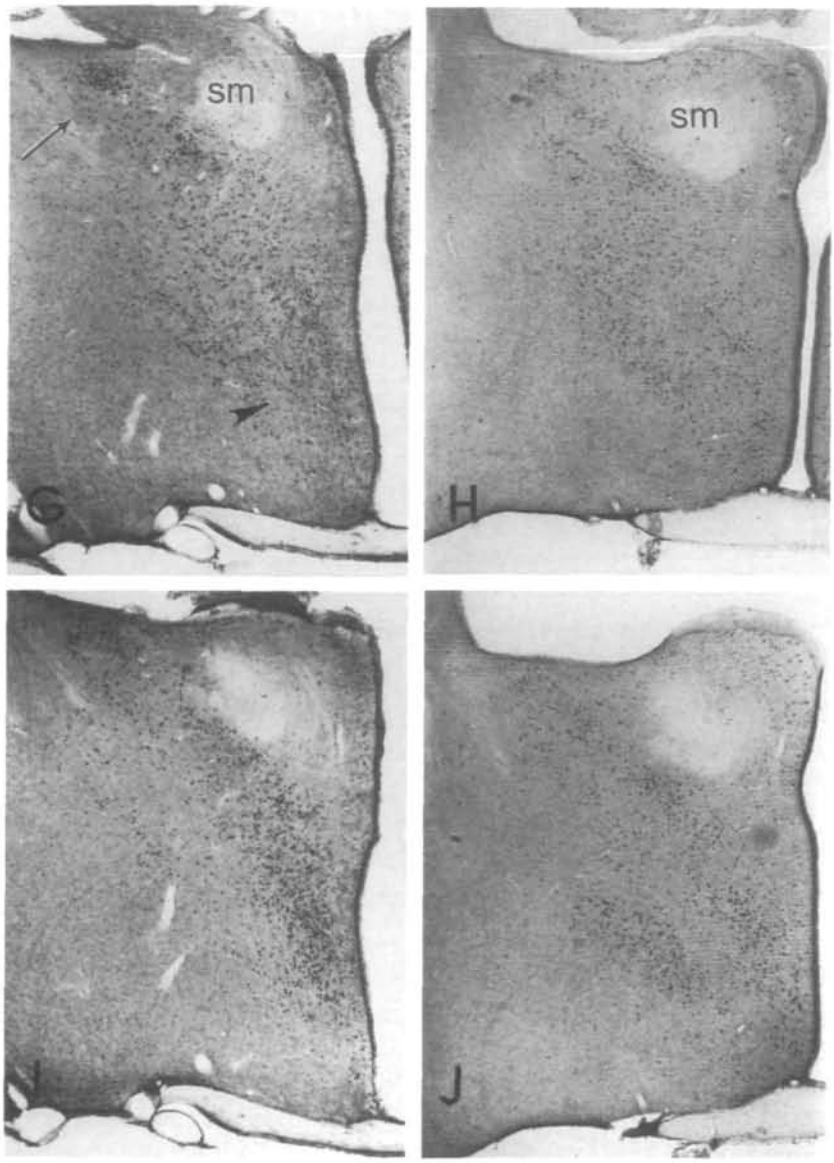

Figure 3 (A–J) Representative sections showing Fos expression in main olfactory and vomeronasal pathways in inexperienced intact- and OLFX-mating animals. (A) Horizontal section through the main olfactory bulb of an intact mating animal showing intense Fos expression concentrated in the granule cell layer, extending into the glomerular layer. (B) Similar section from an OLFX-mating animal showing lack of Fos expression in the MOB cellular layers. (C) Coronal section through the anterior cortical nucleus of the amygdala (ACN: arrowhead) of an intact-mating animal at the level of the rostral anteromedial amygdala (MeA) showing Fos expression. (D) Coronal section through the ACN of an OLFX animal showing little or no Fos expression. (E) Coronal section through the caudal posteromedial amygdala of an intact-mating animal with the two characteristic clusters of activated nuclei (arrowheads) in the dorsal part (cMePd), also showing the PLCN. (F) Similar coronal section from an OLFX-mating animal showing Fos expression in the cMePd and the PMCN, but not in the PLCN. (G) Coronal section through the rostral part of the posteromedial BNST (Pm in Figure 1; arrow) and the MPOA (arrowhead) from an intact-mating animal showing intense Fos expression in both areas. (H) Similar section from an OLFX-mating animal showing similar levels of Fos expression (see group-average data in Figure 5). (I, J) Coronal sections through the caudal part of posteromedial BNST in intact- (I) and OLFX- (J) mating animals showing about equal levels of Fos expression (see group-average data in Figure 6). See line drawings (Figure 1) for neuroanatomical boundaries. ot = optic tract, sm = stria medullaris. Scale bar in A refers to all sections as follows: A, B, 800 μm; C, D, 400 μm; E–J, 500 μm.

Central VN pathways

Central VN pathways (AOB, Me and PMCN) showed intense Fos expression in OLFX-mating males (Figure 3B,D,F; see Figure 1 for outlines of neuroanatomical areas) similar to that previously reported in intact-mating males (Figure 3A,C,E; Figure 5). In both cases, and for all these areas, the differences from the appropriate unstimulated controls were significant. Both mitral and granule cell layers of the AOB in mating animals had significantly higher Fos expression than that in unstimulated-OLFX or unstimu-

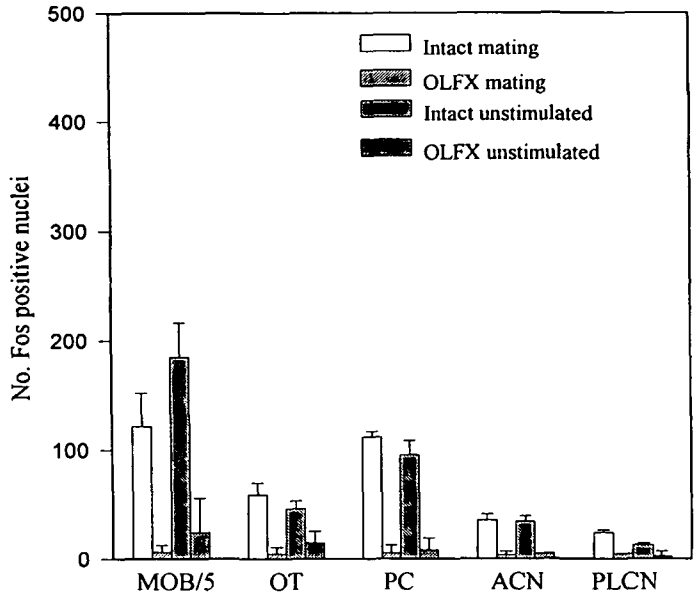

Figure 4 Counts (mean ± SEM) of Fos-positive nuclei in main olfactory pathways of previously inexperienced OLFX animals allowed to mate. Main olfactory pathways, the MOB, OT, PC, ACN and the PLCN, had significantly lower Fos expression in OLFX animals (mating and unstimulated) compared with intact mating and unstimulated animals; but mating animals had no higher levels than the corresponding unstimulated controls (ANOVA). Fos counts of the MOB were divided by 5 to allow counts for all areas to be plotted on the same axes.

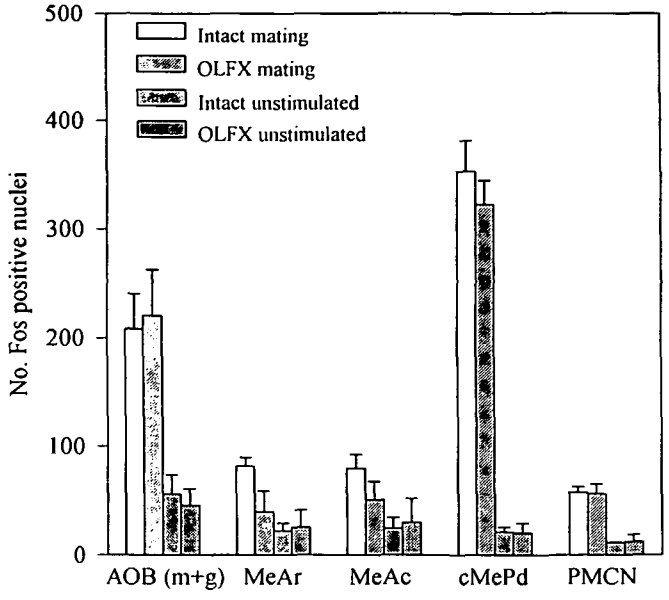

Figure 5 Fos counts in vomeronasal pathways of intact- and OLFX-mating animals. Fos expression in OLFX-mating animals was not significantly different from that in intact-mating animals except in anterior medial amygdala (MeA). The MeA counts are separated into MeAr (rostral) and MeAc (caudal). In MeAr, but not in MeAc, there was a significant reduction in Fos expression in OLFX-mating compared with intact-mating animals. There was a significant difference between mating and unstimulated animals in Me as a whole (ANOVA).

lated-intact controls (Figure 5). Two-way ANOVA showed a significant effect of mating in the mitral cell layer [$F(1,15) = 17.5$, $P < 0.0013$] and the granule cell layer [$F(1,15) = 24.6$, $P < 0.0004$] of both intact and OLFX animals. In the amygdala, there was a significant effect of mating in both MeP [$F(1,15) = 250.67$, $P < 0.0001$] and PMCN [$F(1,15) = 93.28$, $P < 0.0001$]. All OLFX mating animals had the two clusters of Fos-positive nuclei in the caudal dorsal MeP (Figure 3F) characteristic of all mating animals (Figure 3E). There was no significant effect of the olfactory lesion in the AOB, MeP or PMCN when either mating or unstimulated animals were compared with appropriate intact controls. In the MeA there was a significant effect of mating and no effect of OLFX lesion or any significant interaction between mating and OLFX lesion. When the counts were separated into rostral and caudal MeA the results were a little different. The rostral MeA showed a significant effect on Fos expression due to the OLFX lesion [$F(1,15) = 5.69$, $P < 0.034$], a significant increase with mating [$F(1,15) = 19.05$, $P < 0.0009$] and a significant interaction between OLFX lesion and mating [$F(1,15) = 5.96$, $P < 0.031$]. Fos expression in caudal MeA was not significantly reduced in OLFX animals, and data for the combined MeA (rostral + caudal) showed only an effect of mating [$F(1,15) = 8.09$, $P < 0.014$]. Thus, OLFX lesions appear not to affect Fos expression in the AOB, caudal MeA, MeP or PMCN, but there was decreased activation in the rostral MeA, consistent with the known direct olfactory input to this region.

MPOA and BNST

The MPOA and the rostral part of the posterior medial division of the BNST (Figure 3G,I; BNSTpr in Figure 6), previously reported as activated during mating in intact animals (Fernandez-Fewell and Meredith, 1994a), also showed intense Fos expression in OLFX-mating animals (Figure 3H,J). Intact- and OLFX-mating animals had equivalent Fos expression in both these areas (Figure 6), each significantly greater than appropriate unstimulated controls. When compared with intact-mating and intact-unstimulated animals, there was no effect of OLFX lesion, only of mating, in the MPOA [$F(1,15) = 619.57$, $P < 0.0001$] and rostral posteromedial BNST [$F(1,15) = 125.7$, $P < 0.0001$]. The caudal part of posteromedial BNST (Figure 3I,J; BNSTpc in Figure 6), previously reported (Fernandez-Fewell and Meredith, 1994a) to be activated during chemoinvestigatory behaviors and independently of VN sensory input, also had intense Fos expression in OLFX-mating animals. The level of expression was similar to that in intact-mating animals (and similar to that in the occasional VNX animals that mated, or in animals exposed to female hamster vaginal fluid, reported earlier). Two-way ANOVA showed a significant effect of mating [or its associated chemoinvestigatory behavior; $F(1,15) = 176.89$, $P < 0.0001$], but no effect of OLFX lesion in the caudal BNST.

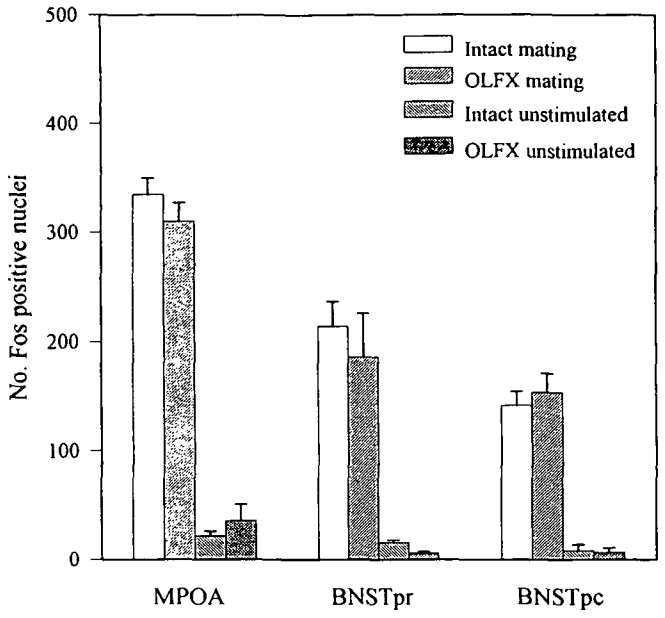

Figure 6 Fos counts in the MPOA and posteromedial BNST of intact- and OLFX-mating animals. There was no significant difference in the Fos expression in any area between intact- and OLFX-mating animals in these regions. For both groups, Fos expression was significantly higher than in unstimulated controls. BNSTpr, BNSTpc = rostral and caudal parts of the posteromedial BNST.

Discussion

We report here a dramatic reduction in Fos expression in the projection areas of the main olfactory system in male hamsters allowed to mate after zinc sulfate olfactory lesions (OLFX). These changes are consistent with the histological picture of sloughing olfactory epithelium and with the functional anosmia demonstrated behaviorally in the same animals. There was essentially no change in the Fos expression patterns in areas thought to be important for mating, on the basis of previous work (Kollack and Newman, 1992; Fernandez-Fewell and Meredith, 1994a; Kollack-Walker and Newman, 1997). These 'mating-related' areas include areas, such as Me, activated by VN input (Fiber *et al.*, 1993; Fernandez-Fewell and Meredith, 1994a), although activation there is not exclusively by VN input. We did not find evidence here that olfactory input contributes to neural activation associated with mating behavior in inexperienced male hamsters, except in the rostral MeA, which does show decreased activation in OLFX animals. It is possible that this MeA input or olfactory activation not evident in the Fos expression pattern contributes, but if so its absence in OLFX animals had no effect on Fos expression in other regions and no obvious effect on mating. Previous behavioral studies have also shown that inexperienced male hamsters do not need main olfactory input to mate normally (Powers and Winans, 1973, 1975; Winans and Powers, 1977; Powers *et al.*, 1979; Pfeifer and Johnston, 1994). Fiber *et al.* (1994) found a reduction in Fos expression in Me and BNST of male hamsters exposed to female hamster vaginal secretion following intranasal zinc sulfate treatment, suggesting that some main olfactory contribution in their intact animals was missing in OLFX animals. We did not find a similar effect here, except in rostral MeA (MeAr).

Olfactory activation of medial amygdala

The activation in the MeAr was significantly lower in OLFX animals than in intact-mating animals. The most rostral part of the MeA receives the only direct main olfactory input in the VN amygdala (Lehman and Winans, 1982; Gomez and Newman, 1992). This is, however, a very small projection compared with the VN projection to the MeA via the AOB. The intra-amygdaloid connections also allow possible interactions between olfactory (ACN and PLCN) and vomeronasal (MeA, MeP, PMCN) amygdala. Lack of these olfactory inputs, both direct and indirect, could account for decreased Fos expression in the rostral MeA. The remaining activation is apparently sufficient for normal mating and, despite a significant projection from the MeA to the MeP (Gomez and Newman, 1992), there was no decrease in the activation of the MeP. Fos expression in the caudal MeP and the PMCN was not different in intact- and OLFX-mating animals. The two clusters of Fos positive nuclei in the dorsal–caudal MeP seen previously in intact and VNX animals that mated (Kollack and Newman, 1992; Fernandez-Fewell and Meredith, 1994a) were also present in OLFX-mating animals. This is consistent with our (Fernandez-Fewell and Meredith, 1994a) and others' (Kollack-Walker and Newman, 1997; Coolen *et al.*, 1997) conclusions that they are related to copulatory performance. The PLCN of the amygdala was the only main olfactory target that showed increased Fos expression in intact-mating animals over unstimulated controls. However, this increase was small and was not seen in animals exposed to pheromonal stimulation by HVF alone (Fernandez, 1994) nor in sexually experienced mating males (Fernandez-Fewell and Meredith, 1994b), suggesting that it may not be due to chemosensory stimulation. It is possible that PLCN activation comes from other cues provided by the female during mating, but it is apparently not necessary for normal mating behavior.

Chemosensory activation of the posteromedial BNST

Fos expression in the caudal posteromedal BNST, which we have previously attributed to chemoinvestigatory activity (Fernandez-Fewell and Meredith, 1994a), was also present in the OLFX animals that mated. Activation there is not solely dependent on VN sensory input since it was present in VNX-stimulated animals (Fernandez-Fewell and Meredith, 1994a), and the present study shows it is not solely dependent on olfactory sensory input either. Since this activation was seen even when animals were exposed to pheromonal stimulation from HVF (Fernandez-Fewell and

Meredith, 1994a), other sensory cues provided by the female during mating must also be non-essential for activation of this region. This activation may be part of the motor or integrative aspects of chemoinvestigatory behavior. It is also possible that the activation in this region may be related to chemosensory input but be capable of being driven by either chemosensory system (olfactory or vomeronasal) separately. We do not know, of course whether the same cells are activated in the different circumstances.

In the rostral part of the posteromedial BNST, Fos expression was seen in all mating animals, both intact and OLFX. We have reported previously that activation of this region is correlated with copulatory performance and that inexperienced animals exposed to HVF do not show the intense activation that is characteristic of all mating animals. This finding is consistent with other studies (Baum and Everitt, 1992; Coolen, 1997) in male rats showing differential activation of rostral and caudal regions of the posteromedial BNST.

Behavioral differences in OLFX animals

Pfeiffer and Johnston (1994) compared chemo-investigatory and copulatory behaviors in both inexperienced and experienced intact and OLFX male hamsters. They found that inexperienced OLFX males spent significantly less time sniffing the genitals of female hamsters and had a significantly higher latency to investigate HVF than inexperienced intact males (Pfeifer and Johnston, 1994). They also had a significantly lower mount latency and mounted more, compared with inexperienced intact male hamsters. We did not measure these behaviors explicitly but if these behavioral differences were present in our experimental animals they were not reflected in the Fos patterns. Our Fos counts in the MPOA and the posteromedial BNST, areas traditionally associated with mating behavior, were not significantly different in the intact-mating compared with OLFX-mating animals. OLFX lesions did not affect other behavioral measures observed by Pfeiffer and Johnston (1994), specifically time sniffing the head or body, duration of mounting or the time the females spent in lordosis.

From previous behavioral studies we know that sexually experienced animals use main olfactory input in the absence of functional VN input during mating. We have preliminary evidence (Fernandez-Fewell and Meredith, 1994b; Fernandez, 1994), to be reported more completely elsewhere, that Fos expression in the olfactory amygdala may be activated to a greater extent by chemosensory input after sexual experience.

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