

Olfactory Bulb Cells Generated in Adult Male Golden Hamsters Are Specifically Activated by Exposure to Estrous Females

Liyue Huang¹ and Eric L. Bittman²

Department of Biology, Center for Neuroendocrine Studies, and Program in Neuroscience and Behavior, The University of Massachusetts, Amherst, Massachusetts 01003

Received August 7, 2001; revised November 10, 2001; accepted November 19, 2001

Two experiments were carried out to test whether cells which are born in adulthood and migrate to the olfactory bulb of adult male golden hamsters are activated during sexual behaviors, to determine the time course over which such responsiveness appears, and to ask whether activation is specific to sexual cues. In the first experiment, adult male hamsters were injected with 5'-bromodeoxyuridine (BrdU, 50mg/kg b.w.) 3 times over the course of one week in order to mark dividing cells. Ten days, three weeks, or seven weeks after the first BrdU injection, the animals were allowed to mate with an estrous female for half an hour before being sacrificed. Confocal analysis of fluorescent immunostaining of BrdU and c-Fos first revealed dual labeled cells in the olfactory bulb 3 weeks after injection of the thymidine analog. In order to determine whether the activation of these newly generated cells is specific to sexual cues, we next compared the incidence of c-Fos expression in newborn (BrdU positive) cells among male hamsters exposed to an estrous female, an aggressive male, a cotton swab containing vaginal secretion from an estrous female hamster (FHVS), a cotton swab containing peppermint, or a cotton swab containing distilled water. In the mitral and glomerular layers of the accessory olfactory bulb, animals exposed to an estrous female had significantly more double labeled cells than did those given other treatments ($p < 0.01$). In the mitral layer of the main bulb, animals exposed to an estrous female had a significantly higher percentage of double labeled cells than those of other groups, except those exposed to an aggressive male ($p < 0.05$). No double labeled cells were seen in medial preoptic area (MPOA), medial nucleus of the amygdala (Me), the bed nucleus of the stria terminalis (BNST), or the hypothalamus. Our results indi-

cate that cells born in adulthood are more responsive to cues arising from estrous females than other stimuli, and thus may participate in sociosexual behaviors.

© 2002 Elsevier Science (USA)

Key Words: olfactory bulbs; BrdU; neurogenesis; c-Fos; sexual behavior.

INTRODUCTION

New cells continue to be generated in adulthood in specific regions of vertebrate brain. In canaries and zebra finches, the seasonal recruitment of new neurons into the hyperstriatum ventrale is believed to be functionally important in the annual reemergence of song (Nottebohm *et al.*, 1986). In adult rodents, cells continue to be generated in the subependymal zone (SEZ) of the lateral ventricle and subgranular layer of the dentate gyrus (Peretto *et al.*, 1999). Cells born in the SEZ travel along the rostral migratory stream to both the main and accessory olfactory bulbs, where they differentiate into interneurons (Lois and Alvarez-Buylla, 1994). In contrast to our understanding of the role of adult-born neurons in the bird song system, however, the functional significance of the incorporation of large populations of new cells into the rodent olfactory bulb is unknown.

We recently documented adult neurogenesis and the existence of a rostral migratory stream in male Syrian hamsters (Huang *et al.*, 1998). Chemosensory control of sexual behavior is particularly well studied in this species. Input from both the main and vomeronasal olfactory epithelia is relayed to a well-characterized set of telencephalic and diencephalic structures in order to coordinate mounting, intromission, and ejaculation (Wood and Newman, 1995). The activation of several of the brain regions critical for male repro-

¹ Current address: Pfizer Global Research & Development, 2800 Plymouth Road, Ann Arbor, MI 48105.

² To whom correspondence and reprint requests should be addressed. E-mail: elb@bio.umass.edu.

ductive behavior, including both the main and accessory olfactory bulbs, the medial nucleus of amygdala (Me), the bed nucleus of the stria terminalis (BNST), and the medial preoptic area (MPOA) is revealed by expression of the protein product of the immediate early gene, *c-fos*, following sexual stimulation (Fiber and Swann 1993; Kollack-Walker and Newman, 1997). Vaginal secretion collected from an estrous hamster (FHVS) is adequate to elicit a similar pattern of Fos expression. Evidence supports participation of both the vomeronasal organ, which projects to the accessory olfactory bulb, and the olfactory sensory epithelium, which projects to the main olfactory bulb, in the activation of Fos expression in the central chemosensory pathway by FHVS (Fernandez-Fewell and Meredith, 1994; Swann and Fiber, 1997; Jang *et al.*, 2001; Swann *et al.*, 2001).

Although our previous work documented stable incorporation of BrdU-ir cells after labeling of cell birth in adult hamsters, we did not examine the time course over which newborn cells migrate to the olfactory bulb. One purpose of the present study was to characterize the migration of BrdU-ir cells at various intervals after BrdU injection in hamsters. More importantly, we sought to gain insight into the possible functional role of olfactory bulb neurons born in adult hamsters by investigating their pattern of Fos expression in response to a variety of stimuli. We found previously that neuronal incorporation is regulated by daylength and gonadal androgen secretion (Huang *et al.*, 1998, 1999). Although these findings are consistent with recent evidence for social and gonadal regulation of the rostral migratory stream in prairie voles (Smith *et al.*, 2001), they do not demonstrate that neurons born in adulthood play a behaviorally significant role. Similarly, anatomical studies which indicate that neurons born in the adult hippocampus extend axonal connections (Staniield and Trice, 1988; Hastings and Gould, 1999; Markakis and Gage, 1999) suggest the potential for functionally relevant roles, but do not document their participation in naturally occurring behaviors. Ideally, we would like to observe effects of selective ablation of cells born in adulthood upon endocrine function or behavior in order to gather evidence for such a role. Unfortunately, such techniques are not yet available. Measurement of the expression of *c-fos* mRNA or Fos protein as a marker of neuronal activation must be carefully interpreted, but this method is the best now available to study the function of cells born in adulthood. We thus applied dual label immunocytochemical methods to evaluate the activation of newborn cells in behaviorally relevant contexts.

MATERIALS AND METHODS

Animals

Male Syrian hamsters (*Mesocricetus auratus*, LVG strain) were born in our laboratory and continuously maintained in 14L:10D. When they were 2.5 months old, all animals were placed in individual cages and given 3 injections of BrdU (dissolved in 0.9% saline made up in 0.07N NaOH; 50 mg/kg body weight, ip) over a course of one week.

In the first experiment, an estrous female was introduced into the males' home cage at the onset of the dark phase of the LD cycle 10 days, 3 weeks, or 7 weeks after the last BrdU injection. Animals were allowed to mate for 30 min, anesthetized with sodium pentobarbital (100 mg/kg), and transcardially perfused with 150 ml of 0.01M phosphate buffered saline (PBS) followed by 300 ml of 4% paraformaldehyde in PBS. Six to nine hamsters were used in each group. The brains were post fixed with 4% paraformaldehyde in PBS overnight and then infiltrated with 30% sucrose. The olfactory bulbs were blocked and sectioned in the sagittal plane at 40 μ m using a freezing microtome. The remainder of the brain was cut similarly but in the coronal plane. Sections were stored in cryoprotectant at -20°C until they were processed for fluorescent immunostaining.

In the second experiment, animals were treated with BrdU as described above but all were sacrificed 7 weeks after the first BrdU injection. On the day of sacrifice, one of the following stimuli was introduced into the home cage during the first four hours of the dark phase, and left with the hamster for 1 h: (1) an estrous female; (2) female hamster vaginal secretion on a cotton swab; (3) an aggressive male; (4) a cotton swab containing peppermint odor; or (5) a cotton swab containing distilled water. Six to nine animals were used in each group except for the water controls, in which 4 animals were used. Behavior for the animals in each group was observed and recorded. Animals were anesthetized and perfused, and their brains were processed as described above.

All animal procedures were reviewed and approved by the institutional animal care and use committee of the University of Massachusetts at Amherst.

Immunocytochemistry

Single immunocytochemical staining. Every sixth brain section including olfactory bulb and regularly spaced coronal sections through the POA, amygdala and hypothalamus were immunostained with either

mouse anti-BrdU (1:1000; Caltag, Burlingame, CA) or rabbit anti-cFos (1:2000, Santa Cruz Biotechnology, Santa Cruz, CA). The staining procedure described by Huang *et al.* (1998) was followed. All steps were carried out at room temperature except where stated otherwise. Briefly, sections were rinsed with PBS followed by incubation in 0.5% NaBH₄ and 0.3% H₂O₂ for 10 min each. Sections to be stained for BrdU were denatured in 2N HCl (30 min at 37°C). Sections were incubated overnight with primary antibody diluted in PBS containing 1% normal goat serum and 0.5% Triton X-100. On the next day, the sections were rinsed with PBS followed by incubation with either biotinylated anti-mouse or biotinylated anti-rabbit secondary antibodies (both from Vector Laboratories, Burlingame, CA, 1:500 in 1% buffered normal goat serum containing 0.5% Triton-X) for 1 h. After rinsing with PBS, the sections were incubated with ABC complex (1:500, Vector) for 1 h. Immunostaining was visualized using 0.03% H₂O₂ and 0.05% 3',5'-diaminobenzidine.

Double immunocytochemical staining. A 1 in 12 series of the brain sections including olfactory bulb were processed for dual fluorescent immunostaining for both BrdU and c-Fos. All procedures were carried out at room temperature unless specified otherwise. Sections were rinsed with PBS first followed by denaturation with 2 N HCl for 30 min at 37°C. After being rinsed with PBS, the sections were blocked with 5% normal goat serum followed by overnight incubation with rat anti-BrdU (1:500, Accurate Chemical and Scientific Corp., Westbury, NY). Tissues were rinsed with PBS and then incubated with goat anti-rat conjugated to FITC (1:100, Jackson Immunoresearch, West Grove, PA) for up to 2 h. The intensity of fluorescent staining was checked under a Zeiss fluorescent microscope in order to optimize the time of incubation in secondary antibody. After BrdU fluorescent immunostaining, the tissues were rinsed with PBS followed by the incubation with a rabbit anti-cFos (1:2000) overnight. On the third day, sections were rinsed with PBS followed by the incubation with goat anti-rabbit conjugated to Cy3 (1:500, Jackson) for up to 2 h, when optimal staining intensity was reached. Sections were then rinsed with distilled water and mounted with Prolong (Molecular Probes, CA). Fluorescent immunostaining was examined under a Zeiss LM510 LSM confocal microscope.

Statistics

Confocal microscopy was performed by an observer blind to the experimental treatment of the animals which generated the sections. Brain regions including MPOA, BNST, Me, main and accessory olfactory bulbs

were scanned for double staining of BrdU and c-Fos. In both main and accessory olfactory bulb, confocal images were taken for the granule cell layer, mitral cell layer and glomerular cell layer. Three sections from the olfactory bulb of each animal, taken at approximately 1-mm intervals and representing the midsagittal, middle parasagittal, and lateral regions of the bulbs, were used for quantification. At least 1000 BrdU-ir cells were scanned in the granule cell layer, 100 in the glomerular layer, and 30–50 in the mitral cell layer of each hamster's olfactory bulb. Double-stained cells were counted and expressed as a percentage of BrdU-ir cells. Analysis of variance was performed to determine the relative efficacy of various stimuli in eliciting Fos expression in BrdU-ir cells.

RESULTS

All males paired with estrous females were observed to complete at least one ejaculatory series before sacrifice in both experiments. In the first experiment, immunocytochemical staining revealed that 10 days after first BrdU injection, the majority of BrdU immunoreactive (BrdU-ir) cells were in the rostral migratory stream (Figs. 1a and 1b). Most of these BrdU-ir cells had elongated nuclei. None of these BrdU-ir cells expressed c-Fos at this time point. Three weeks after the first BrdU injection, about half of the BrdU-ir cells were still in the rostral migratory stream and the subependymal zone of the olfactory bulb, while the rest had migrated into different layers of the olfactory bulb. Few of these BrdU-ir cells were c-Fos positive. Seven weeks after the first BrdU injection, all BrdU-ir cells had migrated into different layers of the olfactory bulb (Fig. 1c). In some individuals, as many as 30% of these cells also expressed c-Fos. Many Fos-ir cells were found in the mPOA, BNST, MeA and hypothalamus, and a few BrdU-ir cells were detected in these areas. Nevertheless, we did not detect any double-labeled cells in the mPOA, BNST, MeA or hypothalamus at any of these survival times (Figs. 2a–2d).

In the second experiment, BrdU-injected hamsters exposed to aggressive males fought with (and generally lost to) these stimulus animals. Hamsters avidly investigated the cotton swabs containing FHVS and peppermint. Exposure of males to estrous females, FHVS, and aggressive males increased Fos immunostaining in the accessory and main olfactory bulbs. As in the first experiment, BrdU-ir cells were predominantly localized in the granule cell layers of the main and accessory olfactory bulbs (Fig. 3). Fewer BrdU-ir cells were found in the mitral and glomerular layers,

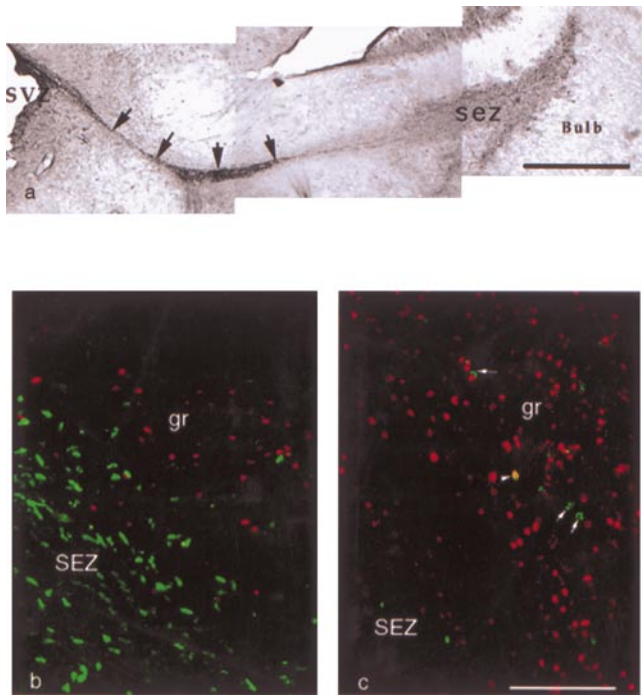


FIG. 1. (a) Montage at low power showing DAB-stained BrdU-ir cells (arrows) in the rostral migratory stream 10 days after the final injection. At this survival time, cells have left the subventricular zone (SVZ, left) but none have entered the olfactory bulb from the subependymal zone (sez, right). Scale bar, 1 mm. (b, c) Dual-label confocal microscopic images illustrating fluorescent immunostaining for (red) c-fos and (green) BrdU in main olfactory bulb of male hamsters (b) 10 days and (c) 7 weeks after injection of BrdU. Hamsters were sacrificed 30 min after introduction of an estrous female. (mi) Mitral cell layer, (gr) granule cell layer. Double labeled cells (yellow) indicated by white arrows in (c). By this time, BrdU-ir cells have left the sez and co-localization is observed. Scale bar in (b) and (c), 100 μm .

and because these layers were difficult to resolve in the accessory olfactory bulb the results were combined for statistical analysis.

In males which mated with estrous females, a mean of 5 to 10% of the BrdU-ir cells in the granule cell layer of both accessory and main olfactory bulb expressed c-Fos (Fig. 4). When the total number of double labeled cells in all layers of either the accessory or the main bulb was compared between groups exposed to various stimuli, animals which were exposed to a female had significantly more activated new cells than any other group except those exposed to FHVS. When we calculated the percentage of BrdU-ir cells that also expressed cFos, we found that pairing with an estrous female was consistently the most effective stimulus in activating neurons born in adulthood. The magnitude of the effect and its statistical significance differed

between the main and accessory olfactory bulbs and between layers within each region of the bulb.

Across all regions of the main olfactory bulb, female hamsters, but not other stimuli, elicited Fos expression in a significantly higher percentage of BrdU-ir cells than did the distilled water cotton swab ($p < 0.05$; Fig. 4). Within the mitral cell layer of the main olfactory bulb, pairing with the female activated Fos expression in a significantly higher percentage of newborn cells than did any other stimulus with the exception of an aggressive male. Although only a few BrdU-ir cells were found in the mitral cell layer (maximum of 50 in the four sections analyzed per animal), up to 30% of the BrdU-ir mitral cells were double-labeled in hamsters exposed to an estrous female. In the granule and glomerular cell layers of the main olfactory bulb, the estrous female was not significantly more effective in eliciting Fos expression in BrdU-ir cells than was FHVS, an aggressive male, or a peppermint-scented swab.

In the combined mitral and glomerular layers of the accessory olfactory bulb, a significantly higher per-

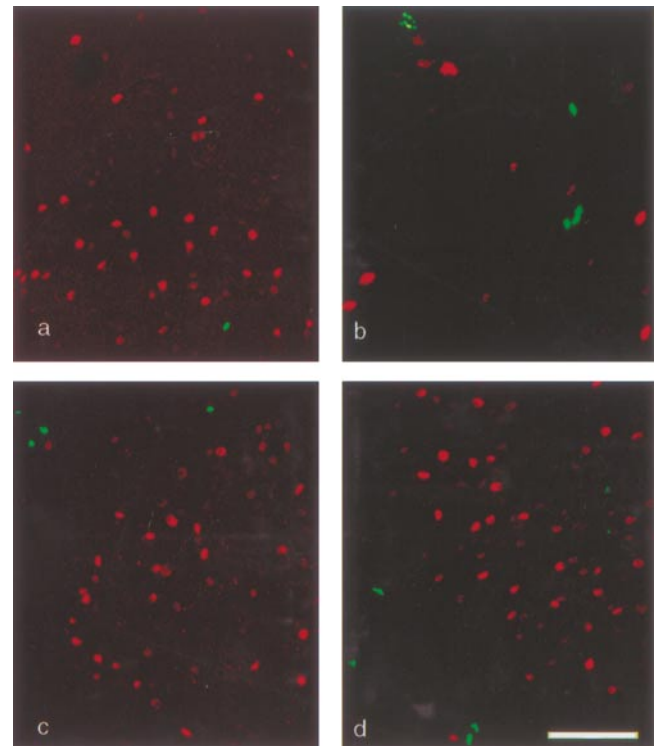


FIG. 2. Confocal images of fluorescent staining of (green) BrdU and (red) c-Fos in (a) preoptic area, (b) BNST, (c) hypothalamus, and (d) medial amygdala of male hamsters sacrificed 7 weeks after BrdU injection and 60 min after introduction of an estrous female. Fos-ir cells were abundant, and a few BrdU-ir cells were found in each of these areas. No cells in any of these regions were double-labeled. Scale bar, 100 μm .

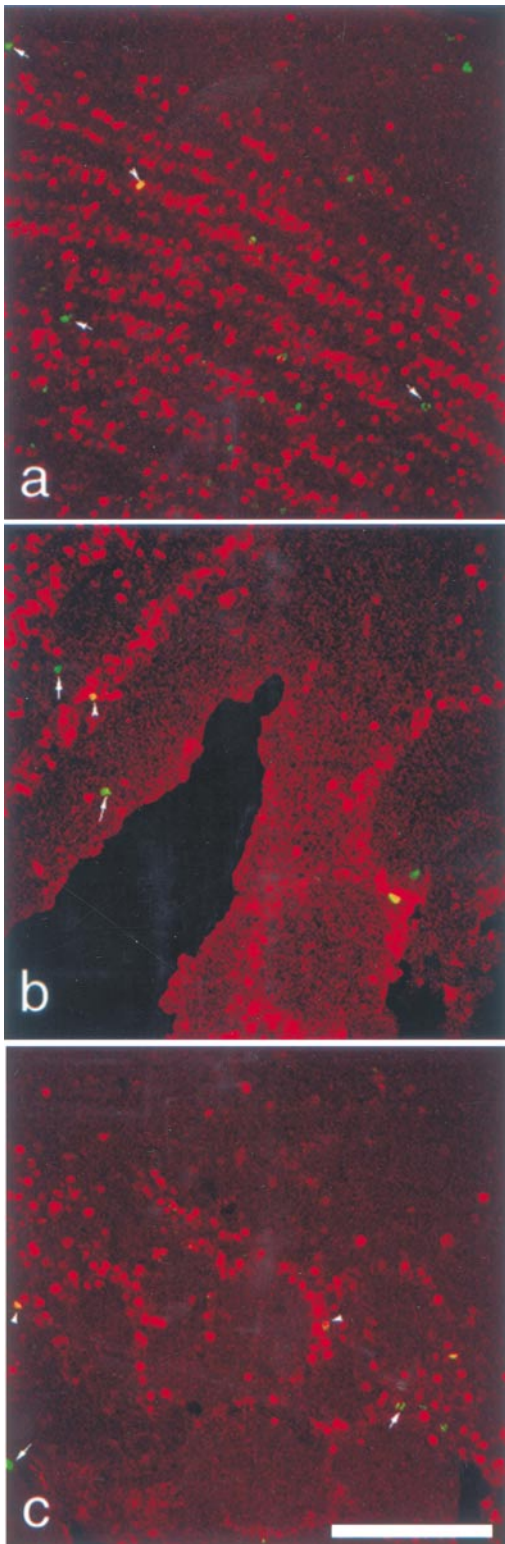


FIG. 3. Confocal image illustrating (green, arrows) BrdU-ir cells, (red) c-Fos-ir cells, and (yellow, arrowheads) double labeled cells in (a) granule, (b) mitral, and (c) glomerular cell layers of the main olfactory bulb in male hamsters exposed to estrous females for 1 h prior to sacrifice. Scalebar, 100 μm .

centage of BrdU cells was double labeled in animals exposed to an estrous female than in those given other stimuli ($p < 0.01$). In the granule cell layer of the accessory olfactory bulb, only FHVS elicited Fos expression in a higher percentage of BrdU-ir cells than did the peppermint odor, and neither of the social stimuli was more effective than the water-soaked swab in eliciting Fos expression.

DISCUSSION

The present results establish that olfactory bulb cells born in adulthood are activated by exposure to estrous females. Other stimuli also trigger Fos expression in some of the cells born in adulthood. Of the stimuli we examined, however, sexual cues were generally the most effective. These findings suggest that neurogenesis and migration in adulthood may play a functionally significant role in maintaining the responsiveness of hamsters to sociosexual cues.

Previous studies have assessed the functional competence of cells born in the adult brain. Okano *et al.* (1993) reported that pentylenetetrazol-induced seizures elicited c-Fos expression in hippocampal cells labeled after injection of ^3H -thymidine in adulthood. The potential for a functional capacity of cells born in the adult hippocampus was also suggested by findings that cells born in adulthood extend axonal projections (Stanfield and Trice, 1988; Hastings and Gould, 1999), and by reports that exposure to enriched environments increases the incorporation of cells born in adult dentate gyrus (Kemperman *et al.*, 1997). Furthermore, the incorporation of newborn cells is modulated by adrenal and gonadal hormones in both the hippocampus (Tanapat and Gould, 1999) and the olfactory bulb (Huang *et al.*, 1998) of rats and hamsters, respectively. In the mouse olfactory bulb, functional relevance was suggested by the finding that deprivation of sensory input reduces the incorporation of cells born in adulthood (Corotto *et al.*, 1994). Although these lines of evidence suggest that such cells could potentially play a physiologically significant function, we believe that the present study is the first to examine the efficacy of naturally occurring stimuli in activating cells born in adult brain.

The difference between the pattern and extent of Fos expression among newborn cells in the main vs accessory olfactory bulb may be related to the differences in activation of neurons in second and third order neurons in pathways activated by sexual vs aggressive stimuli. Although there is considerable overlap of the pattern of activation of Fos-ir in neurons of the amygd-

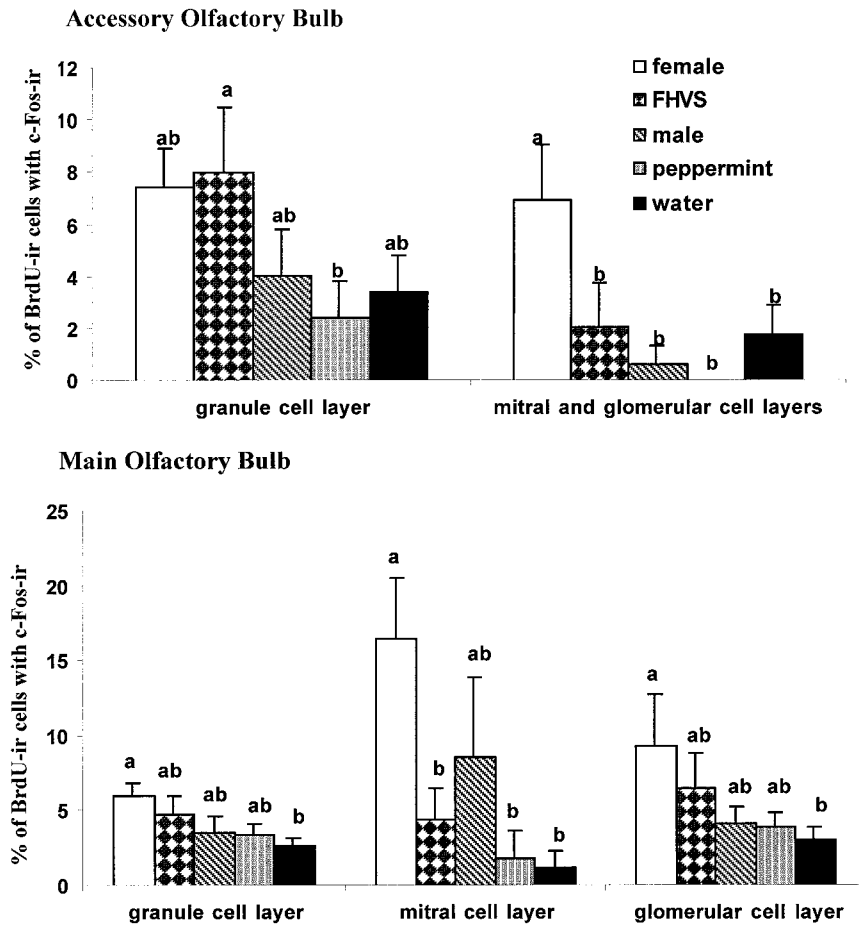


FIG. 4. Percentage of BrdU-ir cells which also expressed c-Fos in confocal sections of granule, mitral, or glomerular cell layers of (top) accessory or (bottom) main olfactory bulb of male hamsters exposed to the indicated stimuli for 1 h prior to sacrifice. Bars with different superscripts differ significantly ($p < 0.05$) in percentage of cells activated. Six to nine animals were used in each group, except for water controls, which included four hamsters. Bars indicate mean \pm SEM.

daloid complex of hamsters exhibiting mating and agonistic behaviors, these stimuli elicit different patterns of Fos expression in the BNST, anterior hypothalamus, preoptic area and periaqueductal gray (Kollack-Walker and Newman, 1995). We found that the pattern of responsiveness to sexual cues extends to the mitral cell layers of both the main and accessory olfactory bulbs. The morphology of the activated cells resembles that of granule cells, however, and it is our interpretation that the activated cells were migrating through the mitral cell layer at the time of perfusion. Although cells in the accessory olfactory bulb are particularly critical in triggering sexual behavior (Winans and Powers, 1977; Powers *et al.*, 1979; Jang *et al.*, 2001), input from the main olfactory bulb may also be necessary for induction of Fos expression in the BNST and MPOA of male hamsters exposed to female pheromones (Swann and Fiber, 1997; Swann *et al.*, 2001).

This is consistent with our finding that new cells are activated by estrous females in both the main and accessory olfactory bulbs.

The present results confirm that many cells of the BNST, Me, and POA express Fos during sexual and aggressive encounters. Our data also confirm that these areas contain cells born in adulthood, although they do not establish whether these cells arise in the SEZ or diverge from the rostral migratory stream to reach these structures. We found no evidence that newborn cells in these areas, in contrast to those that migrate to the olfactory bulbs, are activated by socio-sexual stimuli.

Our observations do not resolve the sufficient stimuli for activation of newborn cells. Stimuli provided by mating with estrous females were marginally more effective than FHVS alone in eliciting c-Fos expression, particularly in the mitral cell layer. This may indicate

that copulation is necessary for full activation of new cells. Alternatively, other sensory cues arising from the female in addition to their vaginal odor may account for the increase in Fos expression in BrdU-ir cells. It is possible, however, that some cells in the olfactory bulbs do not express c-Fos upon activation, or do so at intensities below our level of detection (Dragunow and Faull, 1989). For example, other transcription factors might participate in the response of particular cell types to social stimuli (Keverne, 1999). Thus our methods may underestimate the extent of activation of newborn cells by the stimuli we presented in Experiment 2.

The role of neurogenesis and migration in maintaining sexual behavior requires further investigation. It is well established that the recovery of mounting, intromission, and ejaculatory behaviors takes several weeks after testosterone is administered to castrated hamsters (Christensen *et al.*, 1973). This latency is consistent with our observation that substantial numbers of newborn cells do not arrive in the olfactory bulb until several weeks have elapsed after BrdU injection, and that Fos expression only begins to appear in BrdU-ir cells after three weeks. Does the latency with which cell birth, differentiation, migration, and establishment of connectivity contribute to, or even account for, this delay? The reinstatement of sexual behavior by testosterone may depend at least partially on maintenance or survival and differentiation of cells of the rostral migratory stream, perhaps in addition to effects on other regions (BNST, MPOA; Huang *et al.*, 1999). This hypothesis could be tested by examining effects of arresting neurogenesis by administration of antimetabolic drugs on the maintenance or restoration of male sexual behavior by testosterone.

The present observations also raise the question of whether the proportion of newborn cells which survive is affected by the opportunity to copulate with estrous females. The influence of ovarian hormones on the incorporation of newborn cells into the hippocampus appears to be transient (Tanapat and Gould, 1999). Male rodents experience a pulse of testosterone during and after sexual encounters (Bronson and Desjardins, 1982). This endocrine event, perhaps in conjunction with olfactory activation of newborn cells, may promote the survival of cells born in adulthood. The effects of sexual experience on rates of stable cell incorporation requires further investigation, and the role of the endocrine sequelae of mating behavior on neurogenesis, migration, and apoptosis should be studied.

In conclusion, our findings indicate that cells born in adulthood are responsive to sexual and other social stimuli. The renewal of olfactory bulb cell populations

through the rostral migratory stream may be functionally significant in the maintenance of hamster sexual behavior.

ACKNOWLEDGMENTS

We thank Richard L. Hurlbut for excellent animal care, Vernon Herbert and Jonathan Lawrence for technical assistance, and Dr. J. Bradley Powers for critical reading of the manuscript. Supported by NSF IBN-9817252 and NINDS IF32 NS10691. Portions of this work were presented at the 29th and 30th annual meetings of the Society for Neuroscience.

REFERENCES

- Bronson, F. H., and Desjardins, C. (1982). Endocrine responses to sexual arousal in male mice. *Endocrinology* **111**, 1286–1291.
- Christensen, L. W., Coniglio, L. P., Paup, D. C., and Clemens, L. C. (1973). Sexual behavior of male golden hamsters receiving diverse androgen treatments. *Horm. Behav.* **4**, 223–229.
- Corotto, F. S., Henegar, J. R., and Maruniak, J. A. (1994). Odor deprivation leads to reduced neurogenesis and reduced neuronal survival in the olfactory bulb of the adult mouse. *Neuroscience* **61**, 739–744.
- Dragunow, M., and Faull, R. (1989). The use of *c-fos* as a metabolic marker in neuronal pathway tracing. *J. Neurosci. Methods* **29**, 261–265.
- Fernandez-Fewell, G. D., and Meredith, M. (1994). C-fos expression in vomeronasal pathways of mated or pheromone-stimulated male golden hamsters: contributions from vomeronasal sensory input and expression related to mating performance. *J. Neurosci.* **14**, 3643–3654.
- Fiber, J., and Swann, J. (1993). Pheromones induce c-fos in limbic areas regulating male hamster mating behavior. *Neuroreport* **4**, 871–874.
- Hastings N. B., and Gould, E. (1999). Rapid extension of axons into the CA3 region by adult-generated granule cells. *J. Comp. Neurol.* **413**, 146–154.
- Huang L., DeVries, G. J., and Bittman, E. L. (1998). Photoperiod regulates neuronal bromodeoxyuridine labeling in the brain of a seasonally breeding mammal. *J. Neurobiol.* **36**, 410–420.
- Huang, L., Lawrence, J., and Bittman, E. L. (1999). Cells generated in adulthood are activated during sexual behavior in male golden hamsters. *Neurosci. Abstr.* **25**, 33.7.
- Jang, T., Singer, A. B., and O'Connell, R. J. (2001). Induction of *c-fos* in hamster olfactory bulbs by natural and cloned aphrodisiac. *Neuroreport* **12**, 449–452.
- Kempermann, G., Kuhn, H. G., and Gage, F. H. (1977). More hippocampal neurons in adult mice living in an enriched environment. *Nature* **386**, 493–495.
- Keverne, E. B. (1999). The vomeronasal organ. *Science* **286**, 716–720.
- Kollack-Walker, S., and Newman, S. W. (1995). Mating and agonistic behavior produce different patterns of Fos immunolabeling in the male Syrian hamster brain. *Neuroscience* **66**, 721–736.
- Lois, C., and Alvarez-Buylla, A. (1994). Long-distance neuronal migration in the adult mammalian brain. *Science* **264**, 1145–1148.
- Markakis, E., and Gage, F. H. (1999). Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. *J. Comp. Neurol.* **406**, 449–460.

- Nottebohm, F. T., Nottebohm, M. E., and Crane, L. (1986). Developmental and seasonal changes in canary song and their relationship to changes in the anatomy of song control nuclei. *Behav. Neural Biol.* **46**, 445–471.
- Okano, H. J., Pfaff, D. W., and Gibbs, R. B. (1993). RB and Cdc2 expression in brain: Correlations with ³H-thymidine incorporation and neurogenesis. *J. Neurosci.* **13**, 2930–2938.
- Peretto, P., Merighi, A., Fasolo, A., and Bonfanti, L. (1999). The subependymal layer in rodents: A site of structural plasticity and cell migration in the adult mammalian brain. *Brain Res. Bull.* **49**, 221–243.
- Powers, J. B., Fields, R. B., and Winans, S. S. (1979). Olfactory and vomeronasal system participation in male hamsters' attraction to female vaginal secretions. *Physiol. Behav.* **22**, 77–84.
- Smith, M. T., Pencea, V., Wang, Z., Luskin, M. B., and Insel, T. R. (2001). Increased number of BrdU labeled neurons in the rostral migratory stream of the estrous prairie vole. *Horm. Behav.* **39**, 11–21.
- Stanfield, B. B., and Trice, J. E. (1988). Evidence that granule cells generated in the dentate gyrus of adult rats extend axonal projections. *Exp. Brain Res.* **72**, 399–406.
- Swann, J., and Fiber, J. M. (1997). Sex differences in function of a pheromonally stimulated pathway: Role of steroids and the main olfactory system. *Brain Res. Bull.* **44**, 409–413.
- Swann, J., Rahaman, F., Bijak, T., and Fiber, J. (2001) The main olfactory system mediates pheromone-induced fos expression in the extended amygdala and preoptic area of the male Syrian hamster. *Neuroscience* **105**, 695–706.
- Tanapat, P., Hastings, N. B., Reeves, A. J., and Gould, E. (1999). Estrogen starts a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. *J. Neurosci.* **19**, 5792–5801.
- Winans, S. S., and Powers, J. B. (1977). Olfactory and vomeronasal deafferentation of male hamsters: Histological and behavioral analysis. *Brain Res.* **126**, 325–344.
- Wood, R. I., and Newman, S. W. (1995). Hormonal influence on neurons of the mating behavior pathway in male hamsters. In P. E. Micevych, and R. P. Hammer (Eds.), *Neurobiological Effects of Sex Steroid Hormones*, pp. 3–39. Cambridge University Press. Cambridge, UK.