Comparative Study of Aging in the Mouse Olfactory Bulb

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ABSTRACT
Gene knockout technologies have been used to elevate the mouse as a model species. However, little work has examined age and strain differences in the mouse olfactory system. The present study compared the olfactory bulbs of mature (6 month) and aged (24 month) males of BALB/cBy, C57BL/6J, and DBA/2 strains. Volumes of the glomerular (GLM), external plexiform (EPL), and mitral/granule cell (MIG) layers varied little from strain to strain. Volume measurements increased with age even when corrected for body weight differences. Two nonoverlapping interneuron populations were examined with immunohistochemistry. Staining for the calcium binding protein calretinin varied little between strains, but age-related increases in staining were seen in EPL of C57BL/6J mice. Typical patterns of tyrosine hydroxylase immunoreactivity were observed in all subjects except for old DBA/2 mice, which evidenced considerable staining in submigratory areas. Age-related increases were observed in BALB/cBy and DBA/2 mice but not in the C57BL/6J strain. Glial fibrillary acidic protein staining was similar in old BALB/cBy and DBA/2 mice, with astrocytes in all layers of the bulb, but more concentrated in the MIG. However, C57BL/6J tissue revealed very large astrocytes relatively evenly distributed in all layers. Cell proliferation dropped dramatically with age. Labeled cells could still be observed along the lateral ventricles, but very few were observed within the rostral migratory stream or subventricular zone. Although TUNEL labeling revealed many apoptotic figures in the granule cell layer of young subjects, almost no staining was seen in aged mice. J. Comp. Neurol. 454:361–372, 2002. © 2002 Wiley-Liss, Inc.

Increased interest has emerged in research on the neurobiology of aging. Although structural and neurochemical changes have been studied using a variety of animals, the rapid evolution of mouse genetics calls for more murine models of normal aging and age-related neurodegenerative diseases. Nevertheless, relatively few studies have examined the anatomical, neurochemical, and structural changes that occur in the aging mouse brain. Compounding the problem, many of the existing studies have been carried out in only one inbred strain (largely the C57BL/6J), whereas other inbred and outbred strains have gone unnoticed. Clearly there is a great need for examinations of age- and strain-related changes in the normal, aging mouse brain.

The olfactory system has long been studied as a model for mammalian neural development, maintenance, and degeneration for several reasons. For example, subtle differences in cellular changes are easily discernible throughout the entire system, because the receptor sheet, olfactory bulb, and higher centers such as the anterior olfactory nucleus and the primary olfactory (piriform) cortex all have a limited number of well-defined cell types arranged in a regular, laminar fashion (see, e.g., Shepherd, 1972; Macrides and Davis, 1983; Macrides et al., 1985; Brunjes and Frazier, 1986; Halasz, 1990). The olfactory bulb contains a wealth of neurotransmitter and putative neurotransmitter substances, most confined to specific cell classes, thereby simplifying studies of the expression of neuronal phenotypes. The overall organization of the system is remarkably well conserved among

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species. In addition, a unique capacity for synaptic plasticity has led many researchers to use the olfactory system as a model for phenomena ranging from adult neurogenesis and functional maturation of neurons to long-term potentiation (see, e.g., Brunjes 1994; Philpot et al., 1998a, b; Hatten, 1999; Alvarez-Buylla et al., 2000, 2001; Mouly et al., 2001).

Although substantial numbers of human studies have suggested that functional olfactory deficits increase with age and age-related illnesses (see, e.g., Schiffman, 1979; Schiffman and Leffingwell, 1981; Perl et al, 1992, 1999; Ship and Weilenbach, 1993; Nordin et al., 1996; Moberg and Raz, 1997; Hoffman et al., 1998; Hummel et al., 1998; Larsson and Backman, 1998; Murphy et al., 1998; Geisler et al., 1999; Lehrner et al., 1999; Rolls, 1999), relatively little work has described the normal aging process in animal models. Research from one laboratory (Hinds and McNelly, 1977, 1979, 1981; Kaplan et al., 1985) suggests large age-related changes in rats from 3 to 36 months. Bulb size was found to increase throughout much of the rat’s life, but global decreases in volume with concomitant loss of mitral cells after 24 months were found. During this late phase, mitral cell dendritic size increased, suggesting that the remaining relay neurons attempt to compensate for the reduction in cell number. Electron microscopic studies revealed decreases in mitral cell perikaryal volume, suggesting a breakdown of cellular maintenance. Although these studies in Sprague-Dawley rats suggest that substantial changes do occur with age, other studies in Fischer 344 strains (Baker et al., 1995) suggest fewer differences. As a result, it is obvious that strain differences deserve more thorough examination.

The current work compares the mature and aged olfactory bulbs of three commonly used strains of inbred mice: C57BL/6J, BALB/cBy, and DBA/2. To identify changes in neuroanatomical organization, laminar volume measurements were compared. Changes in neuronal phenotype were assessed by the identification of tyrosine hydroxylase (TH)- and calretinin-containing cells. Astrocytic glial cell populations were compared using an antibody to glial fibrillary acidic protein (GFAP). Patterns of cell production were assessed by the uptake of 2-bromo-5'-deoxyuridine (BrdU) in newly born cells, and patterns of cell death were examined by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL).

**MATERIALS AND METHODS**

**Animals**

Male BALB/cBy, C57BL/6J, and DBA/2 mice (n = 6 animals per age and strain) 6 and 24 months of age (with the exception of two 22-month-old C57BL/6J mice) were obtained from Harlan (Indianapolis, IN) via the National Institute on Aging. Animals were allowed at least 24 hr for acclimation after arrival and were kept in polypropylene cages with food and water ad libidum. Subjects were given a single injection of BrdU (Roche Molecular Biochemicals, Indianapolis, IN; 50 mg/kg), followed by a lethal dose of sodium pentobarbital 2 hours later. Subjects were transcardially perfused with 0.1 M phosphate-buffered saline (pH 7.4), followed by 4% buffered paraformaldehyde. All experiments were endorsed by the Animal Care and Use Committee of the University of Virginia and followed guidelines set by the National Institutes of Health. Every attempt was made to minimize the number of animals required.

**Tissue preparation**

Brains were removed and processed for paraffin embedding. Gross neuroanatomical organization was observed in Nissl-stained series consisting of every tenth 10 μm coronal section. The series was also used to identify the rostral pole of the accessory olfactory bulb, which was employed as the standardized location for subsequent immunohistochemical analyses. Use of such a landmark ensured that all sections examined across ages and strains came from a similar location. Furthermore, to ensure that all sections received identical treatment, all slides contained a representative section from each age and strain. Slides were deparaffinized in xylene, rehydrated through graded alcohols, washed in buffer (pH 7.6), and then incubated in 10% normal serum to prevent nonspecific binding. They were then exposed to one of several primary antibodies overnight at room temperature: 1) To stain proliferating cells, bulbs were exposed to anti-BrdU (1: 100). 2) To examine age and strain differences in interneuron phenotype, we used antibodies to the dopaminergic marker TH (Dialcorin, Stillwater, MN; 1:5,000) and the calcium binding protein calretinin (Chemicon, Temecula, CA; 1:1,000). 3) Distribution of astrocytes was assessed with an antibody to GFAP (Dako, Carpinteria, CA; 1:3,000). After rinsing, slides were incubated in solution containing the appropriate biotinylated secondary antibodies (goat anti-mouse or goat anti-rabbit, Dako; 1:100–200) for 1 hour. After they were rinsed in buffer (pH 7.6), sections were incubated in ABC solution (Vector Laboratories, Burlingame, CA; 1:100) for 1 hour, washed in buffer, and then treated with diaminobenzidine (DAB) and H2O2. The chromagen was allowed to develop for 5–15 minutes, and slides were then rinsed, dehydrated through graded alcohols, cleared in xylene, and coverslipped with DPX (Aldrich Chemical Co., Milwaukee, WI). Primary antisera were omitted for control experiments; no staining was observed under these conditions.

Cell death was assayed with the TUNEL technique using a commercially available kit (TACS 2 TdT Apoptosis Detection Kit; Trevigen, Inc., Gaithersburg, MD). According to the manufacturer’s instructions, on-slide sections were deparaffinized in xylene, rehydrated through a series of graded alcohols, and treated with proteinase K solution for 15 minutes. Next, sections were incubated in a reaction solution containing the terminal transferase enzyme and a mixture of biotinylated nucleotides for 1 hour at 37°C. Afterward, the slides were treated with streptavidin-horseradish peroxidase for 30 minutes. Sections were then treated with a solution containing DAB, and H2O2 was added to develop the chromagen. Control sections treated with DNAase (Trevigen, Inc.) showed diffuse staining of most cells, whereas no labeled cells were observed in sections for which the terminal transferase was not included in the reaction.

**Quantitative analyses**

**Laminar volume measurements.** Nissl-stained series were used to measure age- and strain-related differences in the size of the constituent bulb layers using methods described previously (Brunjes, 1985). For this experiment, three zones were defined: GLM, the glomer-
ular region; EPL, the external plexiform layer; and MIG, the mitral/internal granule cell layers and the subependymal zone (see Fig. 1D). The first section measured was the rostralmost one exhibiting a portion of the GLM; the last section was defined as that with a discernible granule cell layer. The area of each layer was measured in every tenth section. To reduce measurement error, each area was measured three times and a mean computed. Total volume of each layer was determined by 1) multiplying the mean area by section thickness, 2) computing the average volume between adjacent measured sections and then multiplying this number by the number of omitted sections, and 3) adding this number to the volume of the layer in the first section in which it was observed. For each animal, only one bulb was measured. Total bulb volume was computed by summing the volumes of laminae for each bulb as well as volumes/body weight.

**Density of immunolabeling.** Digital images of sections were taken with a color digital video camera (DXC-970MD; Sony) and analyzed with a microcomputer-based image analysis package (MCID 5+; Imaging Research Inc., St. Catherines, Ontario, Canada). Images of sections were acquired, and semiquantitative densitometry measurements of TH, GFAP, and calretinin immunostaining intensity were performed. Variations in illumination across the microscope fields were addressed with a flat-field correction for each separate set of immunolabeled sections. Medialmost quadrants of the GLM, EPL, and MIG were outlined, and the average intensity of immunoreactivity was determined. Relative optical densities (ROD) were calculated as: ROD = \log_{10}(\text{maximum possible number of gray levels/observed gray levels}) \times \log_{10}(1/\text{gray level transmittance}).

**Cell death and proliferating cell counts.** For BrdU-immunolabeled and TUNEL tissue, sections representing the midpoint of the rostrocaudal extent of the bulb were selected for measurement. As in immunostaining experiments, slides contained tissues from all ages and strains and were therefore processed simultaneously. For BrdU tissues, a 500 \(\mu\)m \(\times\) 250 \(\mu\)m box was placed around the subependymal layer, the number of positive profiles inside counted three times, and the mean computed. To assess cell death, a box with an area of 500 \(\mu\)m\(^2\) was placed randomly in the interior of the bulb and the number of TUNEL-positive profiles counted. This procedure was repeated three times, and the mean was computed. For both assessments, at least three nonadjacent sections were measured and averaged for each animal (\(n = 4\) for each age and strain).

For all studies, repeated-measures analyses of variance (ANOVA) was performed to test whether the dependent variable varied as a function of age or strain of the animal. Data were analyzed from main effects and interactions. Post hoc comparisons (Tukey’s HSD) were performed to determine specific strain differences between animals in each age group. Single, paired and independent \(t\)-tests were also used when appropriate. Analyses were considered significant at \(P \leq 0.05\). Digital photomicrographs were compiled with Adobe Photoshop 6.0 and Adobe Illustrator.

**RESULTS**

Upon gross dissection, no differences between age groups or strains in bulb size were apparent (see Fig. 1A,B). Furthermore, no differences were observed between left and right bulbs in any subject, mirroring numerous previous studies indicating that there is no laterality in development. Because staining intensity and patterning was similar in left and right bulbs, laminar area measurements, densitometry, and cell counts were taken from only one bulb. Assessments of strain- or age-related effects on each measurement are addressed separately below.

**Laminar volumes**

Two methods were used to analyze data regarding laminar size. In the first, the “absolute” estimates of volume were compared. ANOVA revealed a significant age effect \(F(5,18) = 10.28, P = 8.21 \times 10^{-6}\); see Fig. 1A). Post hoc analysis showed 1) aged C57BL/6J bulbs to be significantly different from their younger counterparts \((P < 0.01)\) and 2) bulbs from aged BALB/cBy and DBA/2 mice to be significantly different from the aged C57BL/6J strain \((P < 0.05\) and \(P < 0.01\), respectively). No difference was observed within the young group or between the aged BALB/cBy and DBA/2 strains. The second analysis, in which potential variations in body size were controlled for by scaling the data by body weight, also revealed substantial differences \(F(5,18) = 5.24, P = 0.004\). Nevertheless, post hoc comparisons indicated that the only significant difference occurred between young and old mice of the BALB/cBy strain \((P < 0.05\); Fig. 1B). An analysis of individual laminae indicated that the differences between aged and young mice were not due to the differential growth or shrinkage of particular bulb regions. When laminar volumes were expressed as percentages of total bulb size, no age- or strain-related differences were observed \(\text{range of } F(5,18) = 1.54–3.96\); for all comparisons \(P > 0.05\); Fig. 1C).

**Cellular phenotypes**

**TH.** TH-immunoreactive (IR) profiles were present in the periglomerular region of all animals. Somata, found around and between glomeruli, were small and darkly labeled. The cells often had substantial and more lightly stained arborizations within glomeruli. The morphology and position of the cells suggest that they are periglomerular interneurons, a finding consistent with many other studies in rodents and other species (Baker et al., 1983; Stone et al., 1991; Baker and Farbman, 1993; Betarbet et al., 1996). Less intensely stained profiles were sporadically observed in the EPL in young mice, with occasional TH-IR fibers appearing superficially in the granule cell layer.

Similar patterns of immunostaining were observed in all young mice (Fig. 2, upper panels). Aged BALB/cBy and DBA/2 mice exhibited both darker staining and increased density of immunoreactive profiles in all layers compared with their younger counterparts (Fig. 2, lower left and right panels) as well as compared with aged tissue from the C57BL/6J strain. Interestingly, intense labeling was present in the internal plexiform and superficial granule cell layers of 24-month-old DBA/2 tissue (Fig. 2, lower right panel). This staining presumably represents staining of noradrenergic centrifugal fibers. The glomerular regions of 24-month-old C57BL/6J mice exhibited staining similar to that of their young counterparts, and no TH-IR centrifugal fibers were seen in these animals (Fig. 2, upper and lower center panels).
ROD measurements were used to confirm quantitatively the age, strain, and layer differences described above (see Table 1). Significant differences were observed in the density of immunostained profiles for each layer: 1) GLM: overall ANOVA \( F(5,18) = 30.85, P < 0.0001 \); significant post hoc comparisons: 24 months BALB/cBy to 24 months C57BL/6J, 24 months C57BL/6J to 24 months DBA/2, all \( P < 0.01 \); 2) EPL: overall ANOVA \( F(5,18) = 3.57, P < 0.05 \); significant post hoc comparisons: aged C57BL/6J and DBA/2 mice (\( P < 0.01 \)), aged BALB/cBy and C57BL/6J animals (\( P < 0.05 \)), and within the DBA/2 strain across ages (\( P < 0.01 \)); 3) MIG: overall ANOVA \( F(5,18) = 3.04, P < 0.05 \); significant post hoc analysis comparisons: adult C57BL/6J and DBA/2 mice (\( P < 0.01 \)), 6 vs. 24 month BALB/cBy mice (\( P < 0.05 \)), and 6 vs. 24 month DBA/2 mice (\( P < 0.01 \)).

Calretinin. Calretinin immunoreactivity, predominantly cytoplasmic, was present in all subjects tested (see Fig. 3). Dark labeling of somata and lighter labeling of neuronal processes were more obvious in the glomerular region, although immunoreactive profiles were observed in all layers of the bulb. The size and position of cells present in the glomerular layer suggested that they represent a subset of periglomerular interneurons, whereas cells in the superficial EPL were bipolar, with slightly larger somas, probably short axon cells (Kosaka et al., 1995). Numerous cells were also observed in the granule cell layer. Analyses of profile density indicated a significant difference in the EPL (\( F(5,18) = 4.48, P < 0.01 \)), resulting primarily from a large difference between young and old mice of the C57BL/6J strain (see Table 1). ANOVA for the GLM and MIG revealed no significant differences.

GFAP. Darkly stained profiles positive for the astrocytic marker were present in all layers of all bulbs tested (Fig. 4; Bailey et al., 1999). Immunoreactive glia had typical asymmetrical, star-like appearances of fairly consistent size within a single bulb. Immunostained profiles were most prevalent in the internal plexiform and granule cell layers, although some were also observed in the subependymal layer of adult subjects.
Fig. 2. Photomicrographs depicting tyrosine hydroxylase immunoreactivity of 6- and 24-month-old mice of the three strains examined. Typical TH immunoreactivity was observed in all young tissues, with no strain differences. With age, TH immunoreactivity increased in BALB/cBy and DBA/2, but not C57BL/6J, mice (see Table 1). Note TH immunoreactivity in the MIG of aged DBA/2 mice, presumably resulting from staining of catecholaminergic centrifugal fibers. GLM, glomerular layer; EPL, external plexiform layer; MIG, combined mitral/tufted and granule cell layers. Scale bar = 50 μm.
Table 1. Age and Strain-Related Changes in Relative Optical Densities of Immunostained Sections

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Layer</th>
<th>Strain</th>
<th>6 Months Mean (SEM)</th>
<th>24 Months Mean (SEM)</th>
<th>ROD</th>
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<td>GLM</td>
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<td>0.250 (0.005)**</td>
<td>††</td>
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<tr>
<td></td>
<td>C57BL/6</td>
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<td>0.189 (0.002)</td>
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<td></td>
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<td>0.242 (0.004)**</td>
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<td>0.169 (0.005)</td>
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<td>0.175 (0.001)</td>
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<tr>
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<td>0.167 (0.001)**</td>
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<td></td>
<td>MIG</td>
<td>BALB/c</td>
<td>0.174 (0.001)</td>
<td>0.179 (0.002)**</td>
<td>††</td>
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<tr>
<td></td>
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<td>0.176 (0.003)</td>
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<td></td>
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<td>0.174 (0.002)</td>
<td>0.183 (0.003)**</td>
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<tr>
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<td>0.211 (0.008)</td>
<td>0.198 (0.005)</td>
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* Asterisks depict significant differences in staining density between ages within strains. †† P < 0.0001; †† P < 0.01. Daggers depict significant differences between strains at 24 months. No interstrain differences were encountered in the 6 month group. 1P < 0.05, †P < 0.01.

GFAP-positive astrocytes appeared larger and less asymmetrical in both the young and the old C57BL/6J subjects compared with the other strains, but the general location of positive cells did not differ among animals (Fig. 4, upper and lower center panels). ROD measurements of immunoreactivity within bulb laminae showed no differences for the GLM [F(5,18) = 2.58] but revealed a significant difference in both the EPL [F(5,18) = 3.46, P < 0.05] and the MIG [F(5,18) = 3.02, P < 0.05]. Tukey’s HSD post hoc tests indicated that the relevant significant differences were between young and aged C57BL/6J mice in both cases (see Table 1).

**DISCUSSION**

As experimental manipulation of the mouse genome becomes an increasingly powerful tool for the creation of animal models of human health and disease, the need for well-defined phenotypes of unaltered inbred strains becomes more important. Changes that occur in the brain during aging are often species, strain, and region specific. In the few studies that have focused on the age-related changes in the olfactory system, most have indicated several potentially important species- and strain-specific differences. For example, differential decreases of volume in the granule interneuron layer and size of mitral cells has been reported for the Sprague Dawley strain of rats (Hinds and McNelly, 1977, 1979, 1981) and for humans (Smith, 1942; Bhatnagar et al., 1987; Kishikawa et al., 1990) but not for the Fischer 344 rat (Forbes, 1984). Also, no age-related changes in TH expression or activity were reported for two strains of C57BL/6 mice and an F1 hybrid Fisher 344/brown Norway rat (Baker et al., 1995). The present study was designed to determine differences in the aging olfactory bulbs of three commonly used inbred strains of mice by examining changes in laminar volume, expression of several neuronal and glial markers, and patterns of cell production and death.

**Changes in bulb size**

Conflicting findings have been reported on the effects of aging on bulb volume. Comparing bulb sizes estimated from protein content, Baker et al. (1995) found no age-related differences in two strains of mice and one hybrid strain of rat. However, Hinds and McNelly’s (1977) careful morphometric study of the rat reported a 50% increase in the volume of the bulb from 3 to 24 months. The data reported above also suggest an age-related increase in total bulb volume for all mouse strains from 6 to 24 months, with an average increase of approximately 18.3%. All bulb layers increased proportionally in volume. The discrepancy between studies remains to be explained, but it could involve differing methodologies or the fact that matching strains for chronological age might not truly represent differences in the physiological aging process. Interestingly, Hinds and McNelly (1977) reported a subsequent proportional decrease in the volume of all laminae from 24 to 30 months. Confirming these results in the mouse might be difficult, insofar as older specimens are difficult to obtain.
Fig. 3. Photomicrographs depicting calretinin immunoreactivity of 6- and 24-month-old mice of the three strains examined. Aged C57BL/6J mice had significantly darker staining in the EPL than their younger counterparts (see Table 1). GLM, glomerular layer; EPL, external plexiform layer; MIG, combined mitral/tufted and granule cell layers. Scale bar = 50 μm.
Fig. 4. Photomicrographs depicting glial fibrillary acidic protein immunoreactivity of 6- and 24-month-old mice of the three strains examined. Staining for the astrocytic marker was similar in the BALB/cBy and DBA/2 strains; most profiles were found distributed in the granule cell layer, with a scattering in the glomerular region. C57BL/6J tissue contained larger astrocytes, with a more dense distribution. Fewer GFAP-IR astrocytes were apparent in aged tissue of all strains for all bulb layers, but the distribution apparent in juvenile tissue remained. C57BL/6J tissue continued to have larger astrocytes than either of the other strains, but density of stained cells in the EPL and MIG layers decreased greatly from densities seen in young mice. GLM, glomerular layer; EPL, external plexiform layer; MIG, combined mitral/tufted and granule cell layers. Scale bar = 50 μm.
Neuronal phenotypes

TH. Although the localization of TH-IR cells in the periglomerular region of all ages and strains was similar to many previous findings characterizing dopaminergic

Fig. 5. Representative sections with BrdU-labeled mitotic cells from the C57BL/6J strain of young (A,C) and old (B,D) mice. Although considerably fewer labeled profiles were seen in older mice, figures observed in more caudal regions of the rostral migratory stream and in the lateral ventricles indicated that cell proliferation was still occurring (E). F: Profile counts emphasized that cell proliferation decreased with age in all strains (*P < 0.05, **P < 0.01). Scale bar in A = 200 μm for A,B; bar in C = 50 μm for C–E.

Fig. 6. Representative sections of a portion of the granule cell layer from young (A) and old (B) mice from the C57BL/6J strain with TUNEL-labeled cells. Arrows show labeled cells. C: The number of TUNEL-labeled cells decreased dramatically with age regardless of strain (**P < 0.01). Scale bars = 50 μm.
phenotypes in rodents (see, e.g., Stone et al., 1991; Baker et al., 1983, 1995; Baker and Farbman, 1993; Betarbet et al., 1996), some age- and strain-related differences were observed. For example, the density of TH immunoreactivity in the glomerular region of all young animals was similar but had increased markedly in the BALB/cBy and DBA/2 strains by 24 months. The C57BL/6J mice examined showed no such increase. However, once again, evidence conflicting with these results has been reported: Baker et al. (1995) found no changes in TH mRNA or protein expression, activity, or localization between two strains of C57BL/6 mice and a hybrid rat strain.

It has been well documented that TH expression is finely regulated by the amount and/or pattern of afferent activity available, with studies of development (see, e.g., Gesteland et al., 1982; Baker and Farbman, 1993; Puch and Shipley, 1999), bulb deafferentation (see, e.g., Nadi et al., 1981; Baker et al., 1983, 1984), and olfactory depriviation (see, e.g., Baker, 1990; Stone et al., 1991; Baker et al., 1993; Cho et al., 1996; Philpot et al., 1997) demonstrating the effect. Perhaps these observations may help to explain the differential TH expression observed both with aging within the strains and between strains at any age, in that they might reflect differences in the configuration of intrabulbar connections or amount of sensory input. Interestingly, periglomerular cells in the BALB/c strain have been reported to lack synaptic connections with olfactory receptor cell axons (White, 1972, 1973) and to differ from the CD-1 strain in bulb TH content and/or dopamine levels in all brain regions (Baker et al., 1988) as well as response to chemical deafferentation (Baker et al., 1988). The present study provides the first evidence of divergent TH levels for the BALB/cBy strain compared with additional common background strains. In addition, dense TH-IR processes in the MIG were observed in the submural regions of aged DBA/2 subjects, but not in their younger counterparts. Presumably, this atypical pattern of TH staining is due to the immunoreactivity of noradrenergic centrifugal inputs, insofar as TH is involved in catecholamine biosynthesis.

**Calretinin.** The subset of interneurons that stained positively for this calcium binding protein remained remarkably constant in all strains and ages. In fact, measurements of ROD revealed only one difference, less immunoreactivity in the EPL of aged C57BL/6J mice (P < 0.05; see Fig. 3, Table 1). The findings suggest that calretinin-immunopositive cells remain stable throughout the life of an animal. It has been speculated that certain calcium binding proteins contribute a degree of cellular stability; such cells are often resistant to neurodegeneration and perhaps are resilient to shifts in chemical phenotype (Hof et al., 1991, 1993; Hof and Morrission, 1991). Indeed, among the three most commonly studied calcium binding proteins (calbindin, parvalbumin and calretinin), expression of calretinin seems to be the least activity dependent in the olfactory system. Experiments using unilateral naris occlusion and olfactory bulbectomy show calretinin immunoreactivity in the bulb and primary olfactory cortex to be relatively stable, despite the impoverished cellular environment that these manipulations create (Philpot et al., 1997; Lim and Brunjes, 1999).

**Glia phenotypes: GFAP**

Comparative studies of strain- or age-related changes in astrocyte populations in the bulb are few (Bailey et al., 1999). In 1984, Weir et al. found a 100-fold increase in the amount of GFAP (per milligram total protein) between 2-week-old and adult rat bulbs. The increase in protein was due to astrocytic maturation and not proliferation, in that cell counts remained the same. In the present study, no difference in the density of immunostaining was observed in any layer across ages, suggesting that the amount of protein present in mouse adults is also relatively constant. These results suggest, therefore, that no significant GFAP-positive astrocyte proliferation or maturation occurs from adulthood to senescence. However, morphological differences were observed: Astrocytes present in both ages of the C57BL/6J strain were noticeably larger and more symmetrical than those seen in the other strains. The functional significance of this difference remains obscure.

**Cell proliferation and death**

The olfactory bulb is a unique and dynamic neural area, in that it experiences continual neural turnover. Cell death is balanced by the lifelong addition of new cells, and synaptic relations must be constantly tempered by the turnover of incoming sensory axons from the olfactory epithelium (Carr and Farbman, 1992; Cummings et al., 1997; Luskin, 1998) and addition of new interneurons from the subventricular zone via the rostral migratory stream (see, e.g., Alvarez-Buylla et al., 2000, 2001). The balance between cell proliferation and cell death is therefore crucial for tissue function. The equilibrium can be skewed by experimentally increasing cell death (Fiske and Brunjes, 2001) or by manipulation of cell survival (see Brunjes, 1994), but rates of cell proliferation seem to be unaffected by experimental challenges (Frazier-Ciernier and Brunjes, 1989).

In the present study, counts of proliferating and apoptotic profiles were the same across both strains and ages. Rates of both processes decreased sharply when aged animals were compared with young counterparts. Similar changes in proliferation rates have been observed in rats (Hinds and McNelly, 1977). Given the finding that bulb volume increased in all strains from 6 to 24 months, one might expect that there would be a decline in apoptotic profiles. Surprisingly, only very small numbers of cells were labeled. If, indeed, cell death occurs by Hebbian mechanisms in the bulb (Fiske and Brunjes, 2001), the results would indicate that by this age bulb circuits have become quite stable. It is quite possible that, with further aging, apoptosis would increase.

**CONCLUSIONS**

The current work provides the first general comparison of mature and senescent olfactory bulbs of three commonly used strains of inbred mice: C57BL/6J, BALB/cBy, and DBA/2. Some features were found not to be affected by either strain or age (e.g., calcium binding protein expression and astrocytic phenotype), some were affected only by age (e.g., rates of cell birth and death slowing in all strains examined), and some showed both strain- and age-related differences (e.g., expression of a dopaminergic phenotype). Perhaps the observed changes allow continued functioning of the olfactory system into senescence; its integrity is important for a host of behaviors, including sexual, social, and ingestive functions. Nevertheless, the findings highlight the potential dissimilarities between animals of di-
verse backgrounds, suggest that divergent mechanisms for maintaining bulbar integrity during aging may have evolved, and serve to caution those with interests in murine models of health and disease that strain and age are important variables to consider.

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LITERATURE CITED


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