Aging-Related Morphological Changes in the Main Olfactory Bulb of the Fischer 344 Rat

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FORBES, W. B. *Aging-related morphological changes in the main olfactory bulb of the Fischer 344 rat.* NEUROBIOL AGING 5(2) 93–99, 1984.—Aging-related changes in several structural characteristics of the main olfactory bulb (MOB) were evaluated using Fischer 344 rats 3, 18, 30 or 36 months of age. Histological examination of the nasal mucosa revealed no evidence of concurrent rhinitis in any of the animals studied. The internal granular layer of the MOB exhibited continual growth, increasing in volume by a factor of 63% over the range of ages studied. The sizes of MOB mitral cell perikarya and nuclei, expressed either as observed cross-sectional areas or as estimated mean volumes, did not change significantly as a function of age. The numbers of mitral cells exhibiting 2 nucleoli in the plane of section decreased from about 22% in the youngest animals to about 4% in the 18- and 30-month old animals and to nil in the oldest animals. These results are discussed in relation to findings of other investigators using Sprague-Dawley or Wistar rats. It is concluded that major structural age changes in the rat MOB are strain dependent.

Strain effect   Cell volume   Mitral cell   Nucleus   Nucleolis   Internal granular layer

THE olfactory system is of particular interest in experimental gerontology not only because of the role of chemosensory deficits in nutritional problems of the elderly [16,20], but also because the main olfactory bulb (MOB) is an appropriate model for the study of the aging process in cortical systems generally (cf., [21]). In view of the wealth of information currently available regarding its peripheral and central connections, the synaptic interactions among its intrinsic elements, and the basic functional characteristics of its principle circuits [22], the MOB constitutes an advantageous system for the study of aging-related changes in cortical structure/function relationships.

In a remarkably thorough series of reports, Hinds and McNelly described aging-related changes in the structural and untrastructural characteristics of the olfactory epithelium and MOB of two substrains of Sprague-Dawley rats 3–36 months of age [6, 7, 8, 9]. They reported that the volume of the internal granular layer of the MOB increased dramatically between 3 and 18–24 months of age and steadily declined thereafter [6]. This general pattern of growth and atrophy was mirrored in many of the parameters they quantified at both the light- and electron microscopic levels, including the volumes of the other layers of the MOB, the volumes of mitral cell perikarya and nuclei, the volume and length of large dendrites in the external plexiform layer, the number and density of perikaryal mitral-to-granule cell synapses, and the number of glomerular axodendritic synapses [6,7]. Moreover, their findings suggested that both growth and atrophy of the MOB and its constituent neural elements were reactive responses to aging-related changes in the number of olfactory receptor neurons [8]. The observa-

tions that both the peripheral and central components of this system undergo substantial growth in the first two years of life are consistent with reports of neurogenesis of olfactory receptor neurons (e.g., [5,15]) and of MOB granule cells (e.g., [1,12]) in adult rodents.

Reports of age-related changes in the olfactory bulb of the Wistar rat suggest that some of the age effects in the Sprague-Dawley rat reported by Hinds and McNelly may be strain-specific. In an early study using Wistar rats, Smith [23] found a 17% increase in the volume of the whole MOB between 3 and 12 months of age, followed by sharply decreased MOB volume at 24 and 36 months of age. However, his finding that the olfactory nerve layer was completely absent either unilaterally or bilaterally in rats of various ages suggests that many of the animals used in that study were subject to some degree of atrophic rhinitis. More recently, Roselli-Austin and Altman [19] found that neither the volume of the internal granular layer nor the average area of mitral cell profiles changed significantly between 3 and 12 months of age in the Wistar rat. Together, these reports raise some doubt as to the generality of Hinds and McNelly's findings to other rat strains.

In the present study, several parameters of MOB growth were measured in Fischer 344 (F344) rats, 3–36 months of age, following the procedures of Hinds and McNelly [6]. This strain is used widely in experimental aging research in part because of the lower asymptotic body weight of the F344 rat in comparison with other strains. Considering that many of the reported age changes in the olfactory system can be characterized as effects on growth of the olfactory epithelium and MOB, the lower rate of somatic growth in the
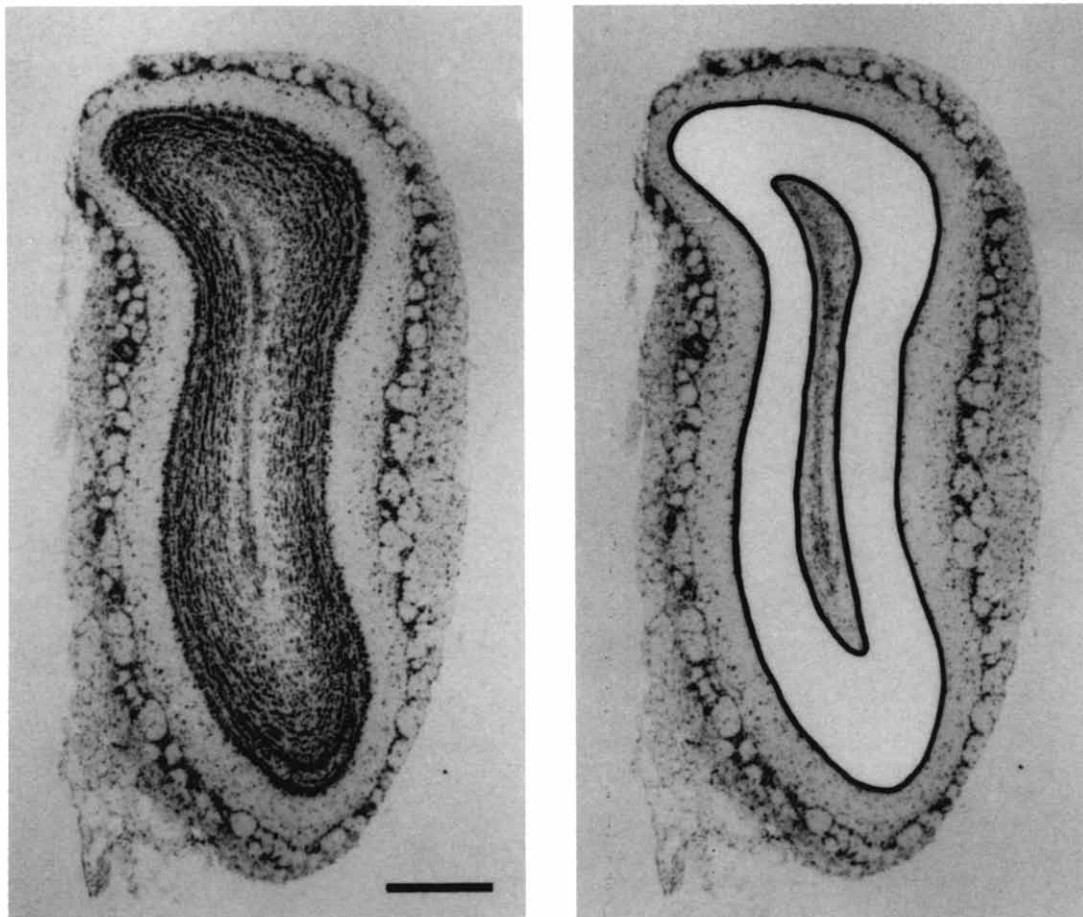
FIG. 1. Coronal section through the main olfactory bulb of the rat. On the right, the area designated as internal granular layer (IGL) for planimetric volume determinations is indicated. Note that, in keeping with the method of Hinds and McNelly ([6], J. Hinds, personal communication), the superficial boundary of the "IGL" is designated as the mitral body layer and thus encompasses the internal plexiform and mitral body layers as well. Calibration 500 μm; 5 μm-thick glycol methacrylate section stained with toluidine blue.

F344 strain might have important consequences for aging in these structures. Furthermore, many American investigators are constrained to study F344 rats by virtue of the fact that this is the only rat strain currently supplied by the National Institute on Aging via its contract colony mechanism. The present study was therefore undertaken to evaluate aging-related structural changes in the MOB of the F344 rat. F344 rats exhibited age patterns in the volume of the internal granular layer and of the mitral cells of the MOB which differed in several important respects from the patterns observed by others using Sprague-Dawley [6,8] or Wistar rats [19,23].

METHOD

Sixteen male F344 rats (COBS®CDF®F344/Crl) obtained from the National Institute on Aging contract colony at Charles River Breeding Laboratories (Wilmington, MA) were used in this study. Four animals were obtained at each of the following ages: 3, 18, 30, and 36 months. Upon receipt from the supplier animals were housed in groups of 2–3 in wire mesh cages with pine shaving bedding. Each animal was

prepared with unilateral electrodes in the right olfactory bulb and left dorsal hippocampus while under barbiturate anesthesia. In addition, a 25 ga. stainless steel cannula was implanted in the left nasal passage just rostral to the main olfactory epithelium. Electrodes and surgical procedures are described in greater detail in a separate report [3].

Approximately 3 weeks following surgery, during which interval electrophysiological data were gathered [3], animals were perfused intracardially, under barbiturate anesthesia, with isotonic saline followed by 1/4 strength Karnowsky's fixative (1% formaldehyde, 1.25% glutaraldehyde in 0.05 M phosphate buffer, pH 7.3). The heads were stored overnight in fixative. The brains were removed and each olfactory bulb was dissected along with a part of the rostral forebrain and blocked in the coronal plane. The nasal fossae were dissected and decalcified using RDO (DuPage Kinetic Laboratories, Naperville, IL). The caudal portion of the nasal fossae and the olfactory bulb blocks were embedded in glycol methacrylate (JB-4, Polysciences, Warrington, PA). Coronal sections of the nasal fossae, 5 μm thick, were taken at several levels through the main olfactory epithelium and stained with hematoxylin and eosin. Coronal sections of the left ol-
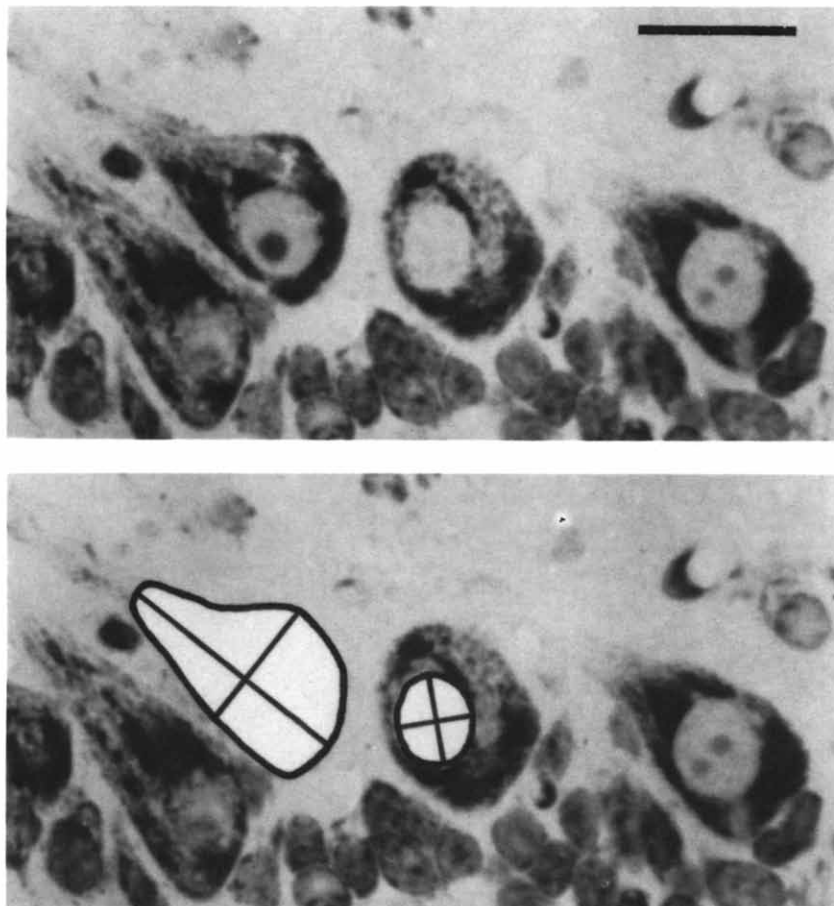
FIG. 2. Mitral cells in the main olfactory bulb of the rat. The major and minor axes of a mitral cell and a mitral cell nucleus are indicated below. In actuality, both cell body and nucleus were measured for each mitral cell selected for study as described in the text. Calibration 20 μm; 5 μm-thick glycol methacrylate section stained with toluidine blue.

factory bulb, 5 μm thick, were taken at intervals of 50 μm through the entire extent of the MOB and stained with toluidine blue.

The volume of the internal granular layer (IGL) of the MOB was determined according to the procedure of Hinds and McNelly [6]. For each animal, 25–30 sections were selected at various intervals encompassing the entire rostrocaudal extent of the IGL. The outline of the IGL in each section was drawn with the aid of a projecting microscope at a magnification of 45× and the cross-sectional area was determined planimetrically. In keeping with the protocol of Hinds and McNelly (J. W. Hinds, personal communication), the mitral body layer (MBL) was taken as the superficial boundary of the IGL (Fig. 1). The intervals between sections selected for analysis were chosen so that the change in the IGL cross-sectional area did not exceed ±20% in successive sections. The volume of the IGL was computed as the sum of the volumes between adjacent sections, estimated by multiplying the means of the two cross-sectional areas by the interval between the sections.

Cross-sectional areas and mean volumes of MOB mitral cell perikarya and nuclei were determined according to

Hinds and McNelly's methods [6], modified slightly to adjust for the thicker sections used in the present study. For each animal, 3 sections were selected for analysis at uniform intervals between the rostral extremity of the anterior olfactory nucleus and the rostral extremity of the MBL. All measurements were performed at a final magnification of 970× using a microscope equipped with a camera lucida attachment. Cells lying within the MBL were identified as mitral cells by the presence of abundant Nissl substance in the perikaryal cytoplasm. The entire MBL in each section was scanned and every 2nd or 3rd mitral cell having distinctly discriminable perikaryal and nuclear boundaries was measured. In this manner, approximately 30 mitral cells in each section were measured. The number of nucleoli in the plane of section was noted for each measured cell.

For each mitral cell studied, the major and minor axes of the perikaryon and nucleus were determined. The perikaryal boundary was defined by the extent of cytoplasmic Nissl substance (Fig. 2). In all subsequent computations, cellular and nuclear profiles were treated as circular, with radii equal to the geometric means of their respective major and minor axes. Thus, for example, the cross-sectional areas of each
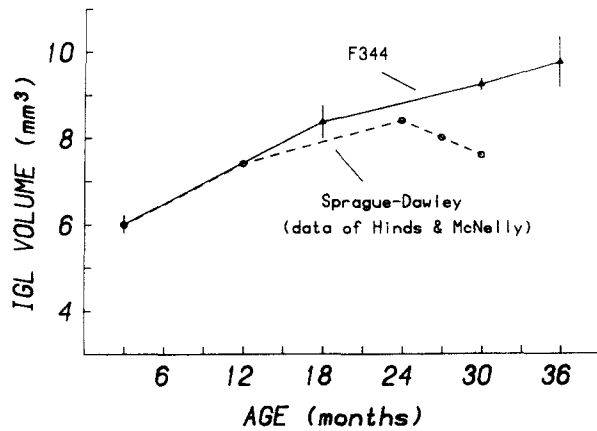
FIG. 3. Age-related changes in the volume of the internal granular layer (IGL) of the rat main olfactory bulb. Data for the Fischer 344 (F344) rats of the present study are given as means±s.e.m.s for each age group (solid line). Data for Sprague-Dawley rats were taken from Hinds and McNelly [6], Fig. 3) and are plotted here as means only (dashed line). IGL volumes were comparable in the two strains up to 24 months of age. At ages greater than 24 months, the Sprague-Dawley rats of Hinds and McNelly exhibited progressively decreasing IGL volumes whereas the F344 rats showed increasing mean IGL volumes up to 36 months, the oldest age studied.

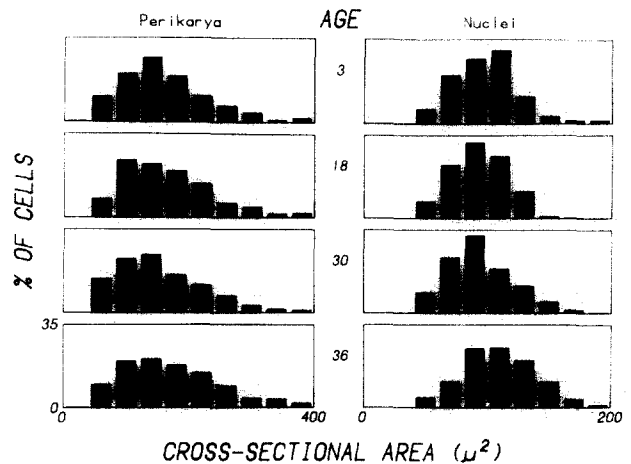CROSS-SECTIONAL AREA (μ^2)

FIG. 4. Relative frequency distributions of observed mitral cell perikaryal and nuclear cross-sectional areas. Each distribution comprises approximately 85 values from each of 4 animals, or about 340 data points. The area measurements shown in these distributions were not corrected for Holmes' effect nor "lost cap" and, as such, constituted the raw data on which various transformations were performed to derive mean perikaryal and nuclear volumes for each animal (see text).

mitral cell and mitral cell nucleus were estimated as the areas of the equivalent circles. Cross-sectional perikaryal area, exclusive of the nucleus, was computed by subtraction. The average cell volume, including the nucleus, for each animal was estimated as the volume of a sphere having a radius equal to the arithmetic mean of the individual cell radii. To estimate the average nuclear volume, the mean observed nuclear radius was computed for each animal, corrected for Holmes' effect and "lost cap" according to the method of Freedman [4], and this corrected mean radius used to compute the volume of an equivalent sphere. Average perikaryal volume, exclusive of the nucleus, was computed by subtraction. It should be noted that the validity of the estimates of cellular and perikaryal size relies, in part, on the assumption that any section through the nucleus passes through the center of the cell (cf., [6]). Although failure of this assumption would result in an underestimation of average cellular or perikaryal size, there is no reason to suspect that such an effect would be age-dependent.

Freedman's correction procedure for the "lost cap" relies on an estimate of the proportion, k/r, of the actual radius for which the profile is not discriminable due to contrast deficiency. In the present study, the value of k/r was estimated according to the formula:

$$k/r = 1 - \sqrt{1-(P_{10}/P_{90})^2} , \qquad (1)$$

where P_{10} and P_{90} are the 10th and 90th percentile observed nuclear radii for a given age group. This method for estimating k/r follows the suggestion of Freedman ([4]; equation 8), though the 10th and 90th percentiles are used here rather than the minima and maxima of the distributions of observed radii to compensate for variability in actual nuclear radius. The mean estimated value of k/r for the 4 age groups, 0.12, was used in all computations of actual nuclear volume. Applying these procedures to Hinds and McNelly's data ([6]; Fig. 6),

estimates of k/r in the range 0.026–0.050 were obtained. These values compare favorably with their own estimate of 0.04, based on a serial reconstruction procedure. The somewhat higher value of k/r for the material used in the present study is in keeping with the greater thickness and lower contrast of the sections in comparison with those used by Hinds and McNelly.

RESULTS

Histological examination of the nasal mucosa revealed no evidence of concurrent rhinitis (cf., [11]) in any of the animals studied. However, several animals of various ages exhibited one or two small adhesions between adjacent olfactory turbinates which were interpreted as indicating a prior history of rhinitis (see Discussion section, below).

Over the 33 month range of ages studied, mean IGL volume increased in approximately linear fashion by a factor of 63%, from 6.0 mm³ in the 3-month old animals to 9.8 mm³ in the 36-month old animals. The effect of age on IGL volume was statistically significant (analysis of variance; $F(3,12)=21.4, p<0.01$). In Fig. 3, group mean IGL volumes for the F344 rats of the present study are shown graphically along with mean IGL volumes for Sprague-Dawley rats (Wisconsin strain) reported by Hinds and McNelly [6]. In comparison, the mean (±s.e.m.) body weights of the animals, recorded on the day of surgery, were as follows: 3 months—197.5±4.7 g; 18 months—394.5±10.3 g; 30 months—399.3±16.1 g; and 36 months—323.5±21.1 g.

In contrast to the dramatic effect of age on IGL volume, none of the indices of mitral cell perikaryal or nuclear size varied significantly across ages. In Fig. 4, estimated cross-sectional perikaryal and nuclear areas are shown as relative frequency histograms for each age group. Analyses of variance based on each animal's mean cross-sectional perikaryal and nuclear areas showed no statistically significant age effect for either parameter, $F(3,12)=1.461$ and 2.550 respec-
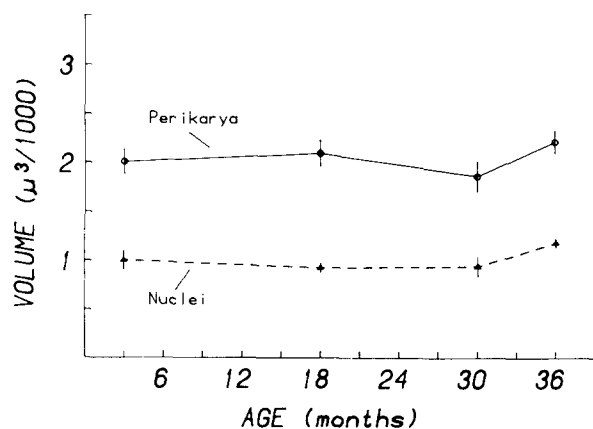
FIG. 5. Estimated volumes of mitral cell nuclei (dashed line) and perikarya (solid line). Data are plotted as means±s.e.m.s. There was no statistically significant age effect for either parameter.

FIG. 6. Comparison of mitral cell parikaryal volume in Fischer 344 (F344) rats (solid line) and two sub-strains of Sprague-Dawley rats (broken lines). Data for Sprague-Dawley rats are taken from Hinds and McNelly ([6], Fig 8; and [8], Fig. 9). Hinds and McNelly's Sprague-Dawley strains exhibited 2- to 3.5-fold increases in mean mitral cell perikaryal volume between 3 and 24 months of age. At 18, 30, and 36 months of age, F344 rats of the present study exhibited group mean perikaryal volumes within ± 10% of the 3-month old group mean. The effect of age was not statistically significant for the F344 rats.

tively; both $p > 0.10$. The grand mean ($\pm$s.e.m.) of the cross-sectional areas of mitral cells, including the nucleus, was 261.5 ± 5.9 μm^2. Group mean mitral cell perikaryal and nuclear volumes are shown in Fig. 5. Neither parameter varied significantly as a function of age (analyses of variance; $F(3,12)=1.291$ and 2.581, respectively; both $p > 0.10$). It should be noted that the latter comparisons are not statistically orthogonal with the respective cross-sectional area comparisons in that the estimates of mean perikaryal and nuclear volume were derived mathematically from the area measurements. In Fig. 6, group mean perikaryal volumes for the F344 rats of the present study are shown along with comparable data of Hinds and McNelly for two substrains of Sprague-Dawley rats [6,8]. Between 3 and 24 months of age, mean mitral cell perikaryal volumes increased by factors of approximately 100% and 200% for the CD and Wisconsin substrains of Sprague-Dawley rats, respectively. In comparison, the three oldest groups of F344 rats all exhibited mean mitral cell perikaryal volumes which were within 10% of the value for the youngest group. The pattern of strain differences was essentially the same for nuclear volume determinations (cf., [6]).

When perikaryal cross-sectional areas were grouped according to the quadrant of the MOB in which each cell lay, a small but consistent trend was evident such that profiles in the dorsal quadrant were, on average, slightly larger, profiles in the ventral quadrant were slightly smaller, and profiles in the medial and lateral quadrants were of an intermediate size. This observation is contrary to that of Hinds and McNelly, who noted slightly smaller profiles in the dorsal quadrant and slightly larger profiles in the ventral quadrant [6]. In view of the fact that their sections were cut in a plane "coronal to the long axis of the olfactory bulb" ([6], pp. 346–347), while in the present study, the plane of section was more nearly vertical, corresponding roughly to the coronal plane of König and Klippel [13], both observations likely reflect the influence of cutting angle relative to the long axis of the cells rather than true regional differences in mitral cell size.

Figure 7 shows the mean numbers of mitral cells exhibiting two nucleoli in the plane of section for the four age groups. This pattern is similar to that observed by Hinds and
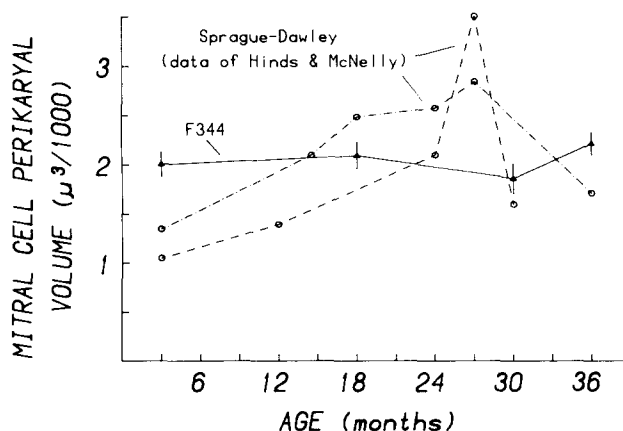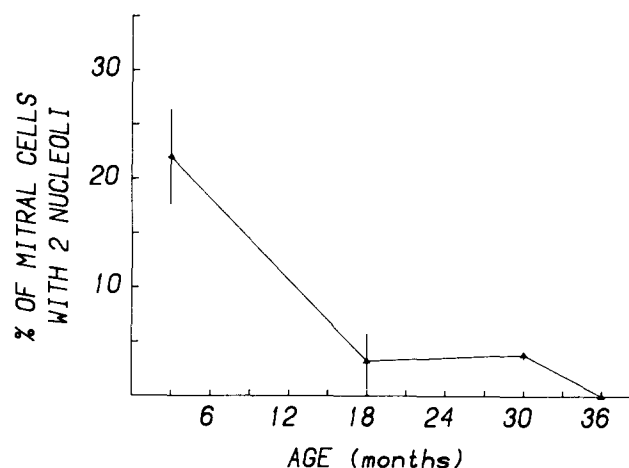

FIG. 7. Percentage of MOB mitral cells with two nucleoli in the plane of section at each age studied. Values are shown as means± s.e.m.s. The number of nucleoli was counted in each mitral cell for which nuclear and perikaryal dimensions were determined.

McNelly [6] in Sprague-Dawley rats (Wisconsin strain), though they reported that the value of this parameter declined to zero by 30 months of age.

DISCUSSION

The results of the present study, together with previous findings of other investigators [6, 8, 19], clearly demonstrate that age changes in the structural characteristics of the rat MOB are strain dependent. Like Sprague-Dawley rats, F344 rats exhibit dramatic growth of the IGL in the first two years of life, but in the F344 rats this growth continues unabated

through the third year as well. Thus, declining IGL volume is not obligatory, even in very old animals. In contrast to the observed age changes in both these strains, Roselli-Austin and Altman [19] found that neither the volume of the whole MOB nor of the IGL changes significantly between 3 and 12 months of age in Wistar rats. It should be noted, however, that a 15–20% change in volume, as seen in F344 and Sprague-Dawley strains over that age range, would be well within the limits of variability they reported. An even stronger strain effect is evident with regard to age changes in the volume of MOB mitral cells. Hinds and McNelly found that, between 3 and 27 months of age, mitral cell volume doubles or triples in Sprague-Dawley strains. Between 3 and 12 months of age, the volume increase is on the order of 30–40% in those strains ([6], Fig. 8; [8], Fig. 9). In contrast, F344 rats exhibit no significant changes in mitral cell volume between 3 and 36 months of age. Likewise, Roselli-Austin and Altman found no significant change in mitral cell size in Wistar rats between 3 and 12 months of age.

It is particularly noteworthy that, in F344 rats, mitral cell volume does not change in register with IGL volume. Hinds and McNelly found that these two parameters, and most of the other light- and electron microscopic features which they quantified, exhibit a rough parallelism both in growth and subsequent atrophy in Sprague-Dawley rats [6, 7, 8]. The present findings indicate that the age changes they observed in IGL volume and in mitral cells, at least during the growth phase, might have resulted from independent age-related processes, since these two age effects are dissociated in F344 rats. Hinds and McNelly have argued that age-related hypertrophy of mitral cells in Sprague-Dawley rats is in reaction to primary age changes in the numbers of olfactory receptor neurons [8], a suggestion which is in keeping with the known proliferative capactiy of olfactory receptor neurons in adult mammals [5,15]. If that were the case, the present findings might imply that neurogenesis among olfactory receptor neurons did not yield an overall increase in the numbers of receptor cells in F344 rats. It may be that increases in IGL volume seen in both strains are in response to turnover or proliferation of MOB granule cells during adulthood (cf., [1,12]) rather than to changes in the numbers of peripheral receptor neurons. There is presently no basis for presuming that these two proliferative processes are coupled to one another in any way. Thus, they might exert parallel influences on mitral cell hypertrophy and IGL growth in Sprague-Dawley strains, but not in the F344 strain.

Although the observed growth of the IGL is unusual in its degree, prior investigators have reported age-related increases in the volume, weight, or linear extent of various brain regions or of whole brain in adult rodents. Such changes are highly region- and strain-specific, however. For example, Hsu and Peng [10] reported volume changes ranging from −2% to +75% in hypothalamic nuclei of Sprague-Dawley rats over 24 months of age in comparison with rats 3.5–5 months old. Even those regions which these investigators found to grow most dramatically, the supreoptic nucleus and medial preoptic area, were found by Lamperti and Blaha [14] to show no age-related size changes in adult golden hamster. Similarly, Brizzee and co-workers [2] reported a 12% increase in whole brain weight between 3.5 and

26–32 months of age in Long-Evans rats, while Roberts and Goldberg [18] reported no significant change in whole brain weight of F344 rats between 2 and 28 months of age.

The observed pattern of age changes in numbers of mitral cells with two nucleoli in the plane of section is remarkably similar to the age pattern of (Sprague-Dawley) rat brain incorporation of [³H]-uridine, an index of the rate of RNA synthesis [17]. Considering that the nucleolus is the site of ribosomal RNA synthesis, age changes in numbers of mitral cell nucleoli might reflect changes in ribosomal gene copy number in these cells. Though there are clearly more direct and, therefore, more valid measures of age changes in the rate of RNA synthesis, the observed age effect on nucleolar numbers has important implications for quantitative analyses of neuron numbers based on nucleolus counts.

Our finding that several animals exhibited small adhesions of the olfactory turbinates is in keeping with the known pathological profile of these Specific Pathogen-Free (SPF) animals. For the past several years, rats and mice maintained in the NIA contract colony have exhibited serological titers to sialodacryoadenitis virus (SDAV; personal communication from Charles River veterinary staff). SDAV is a murine corona virus which is capable of causing moderate to severe rhinitis [11]. In any colony in which SDAV infection is endemic, it is likely that animals become infected shortly after weaning and suffer a brief period of rhinitis followed by reconstitution of the olfactory mucosa. Once infected, animals exhibit serological titers to SDAV throughout the remainder of their lives and are refractory to further infection (cf., [11]). In view of the histological evidence that all of the animals studied were free of concurrent disease, it may be concluded that chronic or recurrent rhinitis was not a factor in the present results.

Although the possibility that differences among various reports of aging in the rat MOB may be due to pathological or methodological factors cannot be ruled out completely, the close similarity of IGL and mitral cell volumes in 3-month old rats of the three strains as well as the differential nature of the strain effect on these parameters argue strongly for the existence of true strain differences. Intraspecific strain effects such as this can be of great value in experimental aging research (cf., [24]). Aging is a multidimensional process which can be manifested in a variety of ways in different species and individuals. One problem facing experimental gerontologists is that of identifying particular age effects which can be experimentally controlled independently of the myriad of other aging-related changes which are manifested concurrently. In this regard, there is a potentially great advantage in comparing closely related strains of animals which show reliable differences in a limited subset of age effects, as appears to be the case in the rat olfactory system.

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