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Expression of Tyrosine Hydroxylase in the Aging, Rodent Olfactory System

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BAKER, H., L. FRANZEN, D. STONE, J. Y. CHO AND F. L. MARGOLIS. *Expression of tyrosine hydroxylase in the aging, rodent olfactory system.* NEUROBIOL AGING **16**(2) 119–128, 1995. – Tyrosine hydroxylase (TH) mRNA, immunoreactivity, and activity were examined as a reflection of dopamine expression in juxtaglomerular neurons intrinsic to the olfactory bulbs of young (6-month-old), middle aged (18-month-old), and aged (25- to 29-month-old) rats and mice. TH expression was maintained at levels observed in young animals in the olfactory bulbs of aged animals from two mouse strains, C57Bl/6JNia and C57Bl/6NNia, and one rat strain, an F1 hybrid between F344 and Brown Norway strains. The parental F344 rat strain exhibited reductions in TH expression of about 20% in 26- to 29-month-old animals as compared to 6- and 18 month-old rats. However, there was significant inter-animal variability. Some aged F344 rats had TH levels that were similar and others had activity levels that were 50% of those in young and middle aged animals. Neither the general condition of the animals nor the presence of adrenal tumors predicted the individuals with reduced TH expression. Olfactory bulb size, estimated from protein content, did not differ between rats and mice of different ages. In addition, expression of olfactory marker protein, a protein found primarily in mature olfactory receptor neurons, also was unchanged indicating the maintenance of afferent innervation. These data suggest that, in contrast to other brain dopamine systems, the expression of the dopamine phenotype is maintained in the aging olfactory bulb.

Denervation Dopamine In situ hybridization Immunocytochemistry Alzheimer's disease Transneuronal regulation Deafferentation

OLFACTORY deficits are a hallmark of both normal aging and some neurodegenerative disorders, such as Parkinson's disease and Alzheimer's disease (AD) (20,23,69). The mechanisms leading to this sensory deficit have yet to be established, but markers associated with degeneration in AD have been observed in the human olfactory system (25,62,75,76,78). Some of the unique properties of the olfactory system could be etiologically significant in this sensory loss. The receptor neurons of the olfactory system, located in the nasal epithelium, are in contact with biological as well as molecular toxins that enter the nares in inspired air and potentially damage these cells. Not surprisingly the olfactory epithelium, unlike other neuronal systems, retains in the adult the ability to replace damaged receptor neurons from progenitor cells located in the basal lamina of the sensory epithelium. When mature receptor cells degenerate as a result of either damage or normal turnover, the progenitor cells divide, mature, migrate within the epithelium, and send an axon through the lamina propria to reinnervate the olfactory bulb (30,31).

As a consequence of the need to respond to the denervation-reinnervation cycle, the olfactory bulb as well as the epithelium, maintains in the adult some properties normally associated with development (58,68,84). One such property is plasticity in the expression of the first enzyme in the catecholamine biosynthetic pathway, tyrosine hydroxylase (TH). The enzyme is found primarily in intrinsic, dopaminergic neurons localized to the juxtaglomerular region of the main olfactory bulb (6). Following either chemical or surgical deafferentation or sensory deprivation of the olfactory bulb, the expression of the dopamine phenotype is profoundly reduced in these neurons (2,3,5,6,7,15, 24,41,61,72,73). Several lines of evidence suggest that the neurons are not lost, but no longer express TH and thus do not synthesize dopamine (2,5,9,41,72,73). In young adult animals, both enzyme and dopamine synthesis resume following reconstitution of the receptor epithelium and reinnervation of the olfactory bulb. In view of the deficits in olfactory function in aging humans, as yet of unknown etiology, one indication of similar age-related changes in plasticity in the rodent olfactory system might be altered steady state expression of the dopamine phenotype.

Brain catecholamine systems, especially the dopamine neurons of the substantia nigra pars compacta (SNc), are known to undergo age-related degeneration in humans, as well as in

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mice and rats which, unlike humans, do not have neuromelanin, a potential neurotoxin, in their catecholamine neurons (11,27,39,42,50,51,55,56,60,63,65,67,74–77,80,82,85). As these changes in expression of the catecholamine phenotype are species, strain and region specific (see, e.g., 1,10,28,67), it is important to examine the olfactory bulb in the species and strain to be used for further experimentation. The few studies that have been done in the rodent olfactory system indicate strain specific differences in several aging-related parameters. For example, loss of afferent innervation, accompanied by decreases in the volume of the internal granule cell layer and size of mitral cells, was reported in aged rats of the Sprague-Dawley (34,35,36) but not the F344 strain (28). Significantly, similar cell loss and volume changes were reported for the human olfactory bulb (13,40,71).

To determine if age-related deficits occur in the rodent olfactory bulb dopamine systems, the current studies examined both changes in afferent innervation and TH expression across age in two mouse and two rat strains. The F344 rat was initially chosen for these studies because of the extensive aging literature available for this strain. However, these rats exhibit extensive tumors (see Results section), including adrenal tumors, which could influence catecholamine expression (86). Thus, a second strain also was analyzed, a cross between the F344 and Brown Norway strains, which does not exhibit these tumors. For comparison, the analysis also included two mouse C57Bl/6 substrains often utilized in aging research (51).

METHOD

Animals

All animals were obtained from the aging colonies maintained by NIA at either Charles River, Wilmington, MA (C57Bl/6JNIA and C57Bl/6NNIA mice as well as F344 Brown Norway F1 Hybrid rats) or Harlan-Sprague Dawley, Indianapolis, IN (F344 rats). Young adult animals were between 6 and 9 months, middle aged were 18 months and aged animals were from 25 to 29 months. Animals, maintained at the Burke Institute animal facility in individually ventilated, hanging, filter-topped cages, were on a 12L:12D cycle with food and water provided ad lib. At the time of sacrifice, the external physical condition was noted for all animals. In addition, all animals were examined for evidence of either degenerative or hyperplastic conditions including tissue necrosis, enlarged organs and tumors including pituitary tumors. Most of the aged F344 rats as previously reported (86) but not F344/Brown Norway F1 rats, exhibited nephropathy and had testicular interstitial cell tumors. The young and middle aged animals did not display these characteristics. Some F344 animals had prominent adrenal tumors as indicated in the results section. Aged animals from both C57Bl/6 mouse strains exhibited alopecia, enlarged seminal vesicles, and a variety of tumors.

Biochemical Measurements

For determination of TH activity, rats and mice were sacrificed by decapitation under CO_2 narcosis. Brains were rapidly removed and the olfactory bulbs isolated, frozen on Dry Ice and stored at -80°C until analyzed. For the F344 rats other tissues also isolated were the *substantia nigra*, *locus ceruleus*, and adrenal glands. TH activity was measured as previously described (9) using 2 mM tyrosine as substrate. Activity is expressed either as nmol DOPA per bulb or nmol DOPA per mg/protein. Olfactory marker protein (OMP) levels were assayed by radioimmunoassay as previously described (4). Protein was determined by the method of Lowry et al. (43).

Immunohistochemistry and In Situ Hybridization

Expression of protein and mRNA levels for TH and OMP were determined by immunocytochemistry and in situ hybridization using previously described techniques (72,73). Briefly, animals received an overdose of pentobarbital and were perfused transcardially with saline followed by 4% buffered (0.1 M sodium phosphate buffer (PB), pH 7.2) formaldehyde generated from paraformaldehyde. Tissue was post fixed for 1 h, cryoprotected in 30% sucrose and 40 μm sections produced on a freezing/sliding microtome. For immunohistochemistry free-floating sections were incubated sequentially with blocking solution for 30 min (1% BSA and 0.2% triton X-100 in 0.1 M PB saline, PBS, pH 7.4), overnight in antiserum (diluted 1/25,000, TH; 1/35,000, OMP), 1 h in biotinylated antirabbit or antigoat IgG and the vectastain ABC elite kit according to manufacturers directions (Vector Laboratories, Burlingame, CA). The chromogen was 0.05% 3-3'diaminobenzidine-HCl and 0.003% hydrogen peroxide. Slides were dehydrated and coverslipped in Permount.

For in situ hybridization, sections were collected in cold 2XSSC (1XSSC is 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0), then incubated with prehybridization buffer for 2 h. Random-primed labelled cDNA probes (10^7 CPM per vial) were added to the vial and hybridization carried out overnight at 48°C. Sections were washed at 48°C in serial dilutions of SSC (2X to 0.1X). After washing in 0.05 M sodium phosphate buffer (pH 7.2), sections were mounted onto gelatin coated slides, dried, and apposed to Kodak XAR-5 film for 1 to 2 days. Slides were then dipped in Kodak NTB-2 emulsion, exposed for 1 to 3 weeks, developed in Kodak D19, fixed, counterstained, dehydrated, and coverslipped. The TH probe was a 0.4-kb EcoRI-KpnI fragment of a rat TH cDNA (16); the GAD probe, a 2.85-kb EcoRI fragment containing the complete coding sequence of a mouse GAD cDNA (37); the OMP probe contained 0.9-kb and 1.4-kb fragments of a rat OMP cDNA (64). The details of the in situ hybridization technique were described in previous reports (72,73).

Statistics

Data was analyzed by Student's *t* test.

RESULTS

Biochemistry

Age-dependent decreases in TH activity occurred only in F344 rats (Table 1). The decrease in three separate experiments

TABLE 1

TYROSINE HYDROXYLASE (TH) ACTIVITY EXPRESSED PER OLFACTORY BULB FROM YOUNG, MIDDLE AGED, AND AGED F344 RATS

Experiment 1		Experiment 2		Experiment 3	
Age in Months (*n*)	TH Activity	Age in Months (*n*)	TH Activity	Age in Months (*n*)	TH Activity
9 (6)	6.28 ± 0.19	6 (6)	4.16 ± 0.22	6 (3)	3.13 ± 0.05
18 (8)	5.99 ± 0.23	18 (6)	3.95 ± 0.17		
29 (7)	4.88 ± 0.43*	27 (6)	3.27 ± 0.19*	29 (3)	2.53 ± 0.19*
29/9	0.78	27/6	0.79	29/6	0.80

TH activity is expressed as nmol DOPA/15 min/bulb ± SEM. *$p \leq 0.05$. *n* = number of animals per group.

TABLE 2

OLFACTORY BULB SIZE IN F344 RATS AS INDICATED BY PROTEIN CONTENT

Experiment 1		Experiment 2		Experiment 3	
Age in Months (*n*)	Protein (mg)	Age in Months (*n*)	Protein (mg)	Age in Months (*n*)	Protein (mg)
9 (6)	5.94 ± 0.22	6 (6)	4.68 ± 0.11	6 (3)	6.80 ± 0.25
18 (8)	5.94 ± 0.18	18 (6)	4.58 ± 0.14		
29 (7)	5.22 ± 0.27	27 (6)	4.49 ± 0.10*	29 (3)	5.67 ± 0.24
29/9	0.96	27/6	0.88	29/6	0.83

Protein levels are expressed as mg protein per olfactory bulb ± SEM. *$p < 0.05$. n = number of animals per group.

averaged about 20%, that is values in aged (27 to 29 months) rats were about 80% of those observed in young (6 to 9 months), or middle-aged (18 months) animals. However, the decreases found were not uniform. Activity in some aged animals was either the same as or higher than that observed in the younger animals. Other aged rats displayed decreases in activity of 50% compared to young animals. Figure 1, a bar graph representing the values for individual animals, shows the larger variability found in aged F344 rats compared to young and middle aged animals. The alterations were not secondary to altered bulb size in F344 rats, as indicated by protein content (Table 2) which did not decrease significantly. Thus, activity differed whether expressed either per olfactory bulb (Table 1) or per mg protein (Table 3). The one exception was in the third group of F344 rats where no difference occurred in TH activity expressed per mg protein possibly because of the small number of animals analyzed (3 per group). A difference was seen when expressed per bulb. No obvious differences were apparent in the condition of the animals (see discussion) exhibiting either high or low TH activity.

To assess whether the variable loss of TH activity in aged animals was accompanied by similar changes in other catecholaminergic systems TH activity, in one group of animals, was measured in two other brain regions: the *substantia nigra* and the *locus ceruleus*, as well as in the *adrenal medulla*. TH activity uniformly decreased in the *substantia nigra*, was not significantly altered in the *locus ceruleus* and increased in the adrenal medulla (Table 4). Although some animals displayed obvious

TABLE 3

TYROSINE HYDROXYLASE (TH) ACTIVITY EXPRESSED PER MG PROTEIN IN OLFACTORY BULTS FROM YOUNG, MIDDLE AGED, AND AGED F344 RATS

Experiment 1		Experiment 2		Experiment 3	
Age in Months (*n*)	TH Activity	Age in Months (*n*)	TH Activity	Age in Months (*n*)	TH Activity
9 (6)	0.80 ± 0.03	6 (6)	0.88 ± 0.04	6 (3)	0.46 ± 0.02
18 (8)	0.76 ± 0.02	18 (6)	0.86 ± 0.03		
29 (7)	0.70 ± 0.03*	27 (6)	0.73 ± 0.04*	29 (3)	0.45 ± 0.04
29/9	0.87	27/6	0.83	29/6	0.98

TH activity is expressed as nmol DOPA/15min/mg prot. ± SEM. *$p \leq 0.05$. n = number of animals per group.

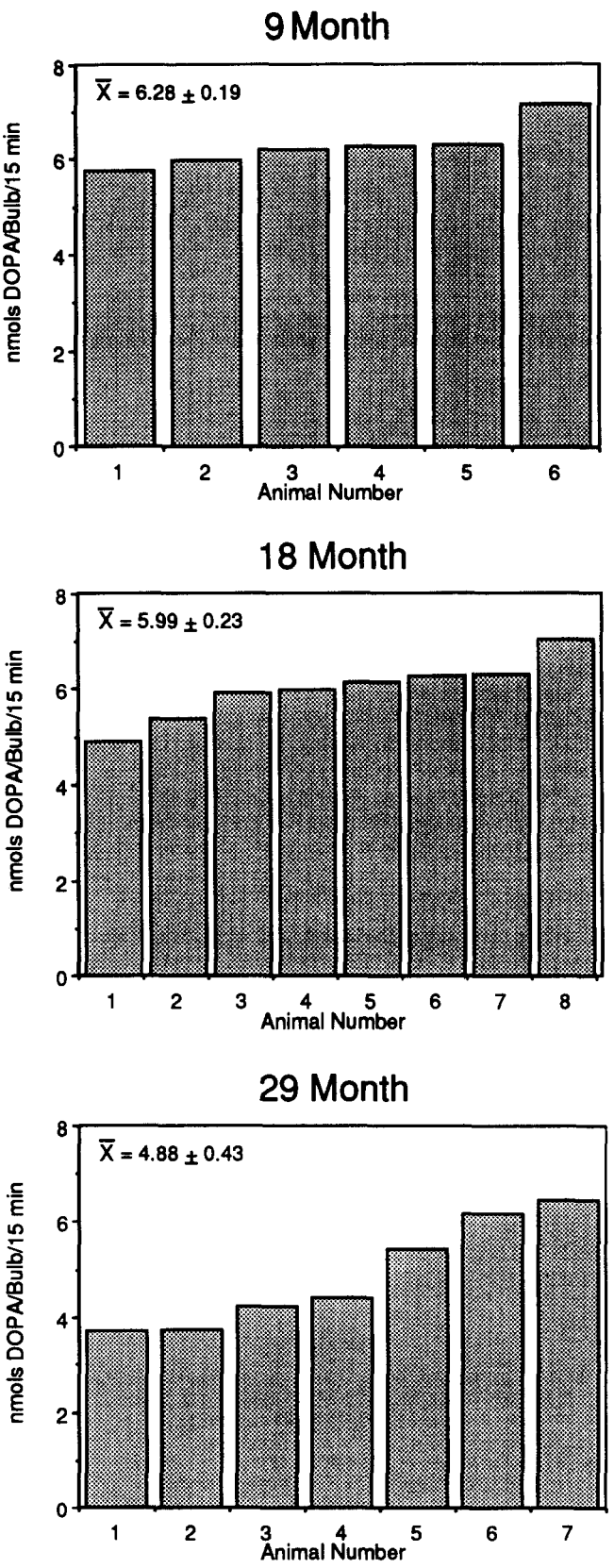

FIG. 1. Tyrosine hydroxylase (TH) activity in individual F344 rats at 3 ages, 9-, 18-, and 29-months. Note the larger variability in activity in aged animals. The mean data are the same as those indicated in Table 1, Experiment 1.

TABLE 4

TYROSINE HYDROXYLASE (TH) ACTIVITY IN OLFACTORY BULBS, SUBSTANTIA NIGRA, LOCUS CERULEUS, AND ADRENAL GLANDS FROM YOUNG, MIDDLE AGED, AND AGED F344 RATS

Age in Months	Olfactory Bulb	Substantia Nigra	Locus Ceruleus	Adrenal Gland
6	4.16 ± 0.22	7.31 ± 0.31	1.18 ± 0.07	31.98 ± 1.67
18	3.95 ± 0.17	7.06 ± 0.22	1.00 ± 0.03	38.78 ± 1.16
27	3.27 ± 0.19*	5.80 ± 0.02*	1.02 ± 0.04	56.37 ± 4.66*
27/6	0.79	0.80	0.90	1.76

TH activity is expressed as nmol DOPA/15 min/region ± SEM; $n = 6$ for all groups but aged adrenals where $n = 4$. *$p < 0.05$.

TABLE 6

TYROSINE HYDROXYLASE (TH) ACTIVITY IN OLFACTORY BULBS FROM YOUNG, MIDDLE AGED, AND AGED C57Bl/6 NIA MICE

Experiment 1		Experiment 2		Experiment 3	
Age in Months (*n*)	TH Activity	Age in Months (*n*)	TH Activity	Age in Months (*n*)	TH Activity
6 (5)	2.18 ± 0.16	6 (6)	2.82 ± 0.08	6 (12)	1.71 ± 0.05
18 (5)	2.08 ± 0.07	18 (6)	2.83 ± 0.08	18 (5)	2.05 ± 0.11
26 (4)	1.94 ± 0.10	24 (6)	2.76 ± 0.12	25-28 (4)	1.79 ± 0.12
26/6	0.89	24/6	0.98	25-28/6	1.05

TH activity is expressed as nmol DOPA/15 min/bulb ± SEM. *$p \leq 0.05$. n = number of animals per group.

adrenal tumors, in others the adrenal glands appeared normal and had typical protein levels. The adrenal TH activity values presented in Table 4 do not include the data from the animals with tumors. No correlation existed between TH activity in the olfactory bulb and the presence of adrenal tumors.

In contrast to the F344 rats, F344/Brown Norway F1 rats did not exhibit any age-dependent changes in TH activity whether the data was expressed either per olfactory bulb or per mg protein (Table 5). The oldest animals examined were 28-months-old, about the same age as the F344 rats which did exhibit changes.

Age-dependent changes in TH activity also did not occur in the olfactory bulbs of two mouse strains, C57Bl/6NNIA and C57Bl/6JNIA mice. The findings in the former group were replicated in 3 separate experiments (Table 6 and Table 7). As indicated by protein content, olfactory bulb size remained constant (data not shown) and, therefore, TH activity also was unchanged when expressed per mg protein (see for example Table 7).

Immunohistochemistry and In Situ Hybridization

TH protein and mRNA levels also were assessed using a specific antiserum and cDNA probe, respectively. As previously described (4), TH is found in small granule-type cells, juxtaglomerular cells, which surround the glomeruli of the main olfactory bulb. These neurons send their dendritic processes into the glomeruli where they receive innervation from receptor afferent processes and make reciprocal synaptic contacts with mitral cell dendrites. The distribution of TH protein and message did not differ between young, middle- aged, and aged F344 rats. However, TH protein and mRNA levels in the aged (26-to 29-month) F334 rats exhibited the same variability as found for TH activity, that is, expression decreased substantially in some animals and looked relatively normal in others. Figure 2 illustrates the differences observed in TH expression between 6-month-old rats and some 29-month-old rats. There is a general decrease in the intensity of immunostaining and a number of silver grains representing TH protein and mRNA, respectively. At higher magnification, shown in Fig. 3, the differences in the number of TH neurons can be better appreciated. Also apparent is a decreased TH fiber density within the glomeruli of the older animal. In other aged animals, there was little or no change in either TH immunoreactivity or message levels.

Afferent innervation in the F344 rats was assessed with an antiserum to olfactory marker protein (OMP), a protein expressed primarily in olfactory receptor neurons. OMP immunoreactivity did not show dramatic decreases in aged as compared to either middle-aged or young rats (Fig. 4), a finding confirmed biochemically using a radioimmunoassay for OMP (6-month, 17.76 ± 1.08; 29-month, 15.32 ± 2.28 μg/bulb ± SEM; $n = 3$/group; $p = 0.386$).

Similar studies performed in both mouse strains confirmed the biochemical data. TH protein and message levels were unchanged with age (Fig. 5, A-D). mRNA levels for glutamic acid decarboxylase (GAD) also were unchanged (Fig. 5 E & F). In addition, OMP immunostaining remained constant (data not shown). As previously reported (38), OMP message could be detected in the nerve fiber layer of the olfactory bulb, a region which contains no neuronal cells but only the axonal terminations of olfactory receptor neurons (Fig. 6). The distribution of OMP mRNA was similar in young and aged animals.

DISCUSSION

Although age-related alterations in the expression of the catecholamine phenotypes have been assessed in many brain regions (60), these parameters have not been examined in the olfactory

TABLE 5

TYROSINE HYDROXYLASE (TH) ACTIVITY IN OLFACTORY BULB FROM YOUNG, MIDDLE AGED, AND AGED F344/BROWN NORWAY F1 HYBRID RATS

Age in Months	TH Activity/Bulb	TH Activity/mg Protein
6	3.63 ± 0.17	0.659 ± 0.04
18	3.36 ± 0.19	0.590 ± 0.03
28	3.61 ± 0.16	0.696 ± 0.03
28/6	0.99	1.06

TH activity is expressed as nmol DOPA/15 min/bulb ± SEM. TH activity is expressed as nmol DOPA/15 min/mg protein. All groups contained six animals.

TABLE 7

TYROSINE HYDROXYLASE (TH) ACTIVITY IN OLFACTORY BULBS FROM YOUNG AND OLD C57Bl/6J NIA MICE

Age in Months (*n*)	TH Activity/Bulb	TH Activity/mg Protein
6 (6)	0.691 ± 0.032	0.486 ± 0.021
25 (4)	0.818 ± 0.050	0.533 ± 0.031
25/6	1.18	1.10

TH activity is expressed as nmol DOPA/15 min/bulb ± SEM. TH activity is expressed as nmol DOPA/15 min/mg protein. n = number of animals per group.

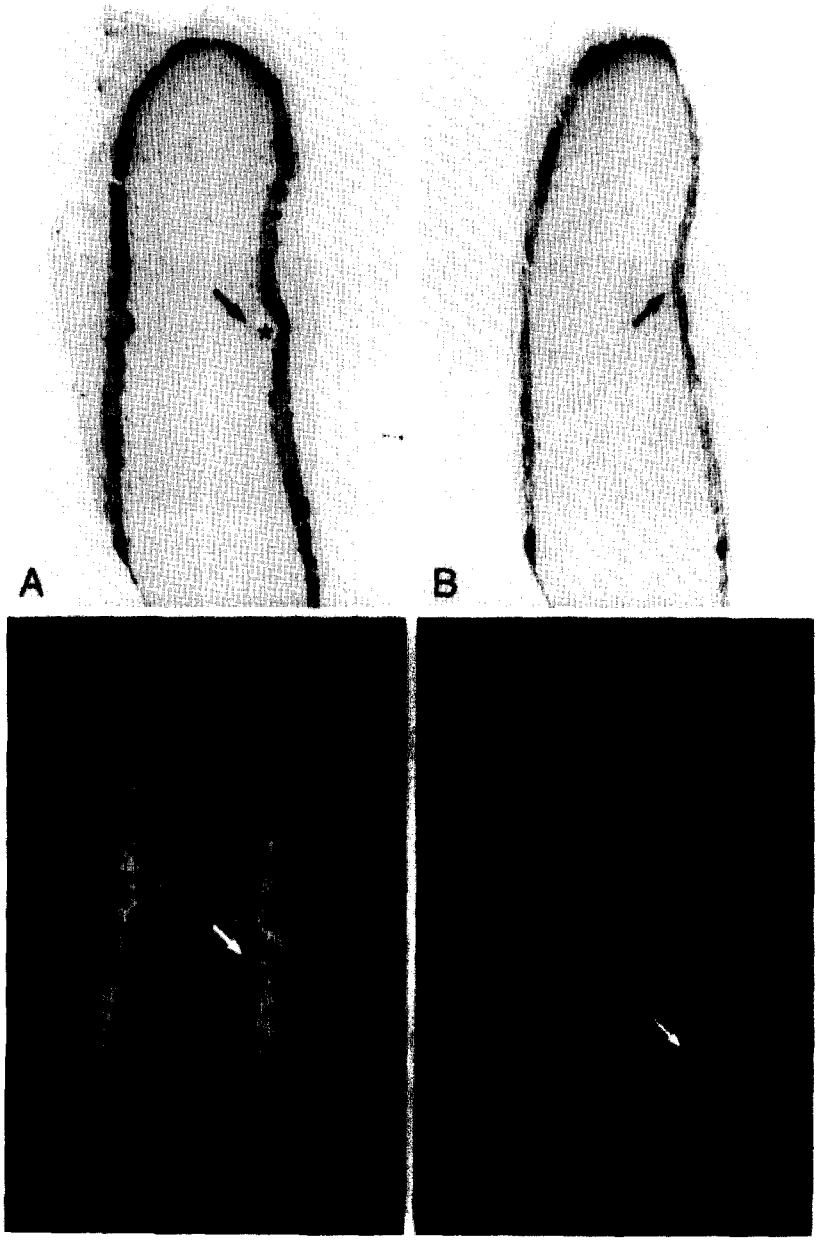

FIG. 2. Low power photomicrographs of TH protein (A and B) and mRNA (C and D) in F344 rats. A and C are representative sections from a 6-month-old animal. B and D demonstrate the decrease in TH expression in one 29-month-old animal. The asterisks indicate needle holes used to distinguish young from aged animals which were processed simultaneously for comparative purposes. Arrows indicate regions illustrated at higher magnification in Fig. 3. Bar = 400 μm.

bulb. The data presented here demonstrate within and between strain differences in TH expression in the aging rat and mouse olfactory bulb. TH activity, immunoreactivity, and mRNA levels were not decreased in the mouse strains investigated in these studies. Similarly, F344 Brown Norway F1 hybrid rats also did not show any age-related changes in TH expression in the olfactory bulb. In contrast, some rats of the F344 strain did show an age-related decrease in TH expression which was not demonstrable at 18 months but was apparent by 26 months.

Several potential mechanisms could underlie the species and strain specific differences in the expression of the dopamine phenotype during aging. First, as has been shown for other parameters, species and strain differences in aging occur in the olfactory system. Mitral cell number decreased in one rat strain (Sprague-Dawley, Wisconsin) (34,35,36) and in humans but not in another rat strain (F344) (28). Changes also occurred in afferent innervation in some strains, a pattern also observed in humans (34,35,36,71). The altered dopamine expression in the

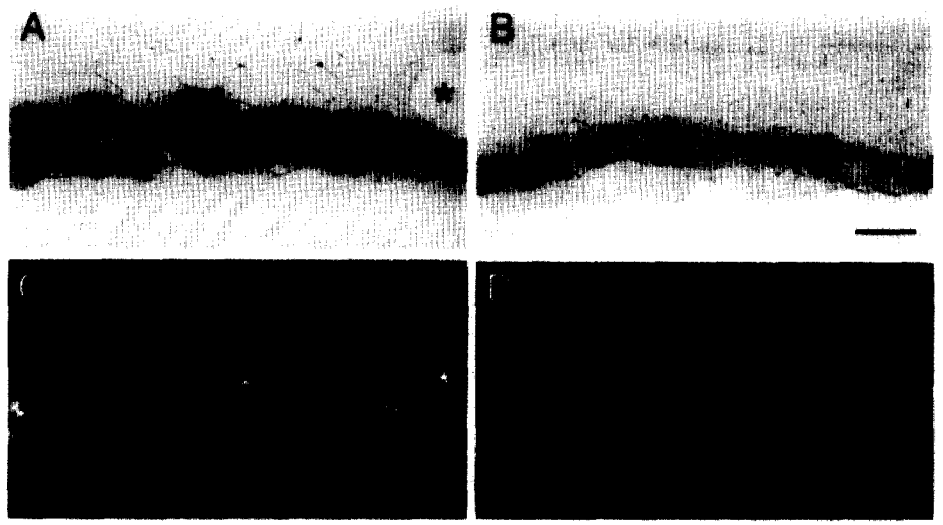

FIG. 3. High power photomicrographs of TH protein (A and B) and mRNA (C and D) in F344 rats. There appears to be a decrease in both the number of cells containing TH message and protein as well as in the intensity of immunostained fibers within the glomeruli. Bar = 120 μm.

F344 rat could be one manifestation of these species differences. The random nature of the decrease in TH expression in individual F344 rats precludes easy analysis of the underlying mechanisms because it is not possible to predict a priori which animals will exhibit the deficit. The consistency of the effect, however, was demonstrated by the finding that, in three replicate experiments, a similar number of animals showed the loss of TH expression and the degree of change was the same (a reduction of approximately 20%).

Second, the age-related differences in TH activity could reflect variations in the rate of aging between the two rat strains. The F1 hybrid F344-Brown Norway rat appeared much healthier than the F344 rat at the same age (about 28 months). The longer life-span of the F344 Brown Norway F1 hybrids (Haz-

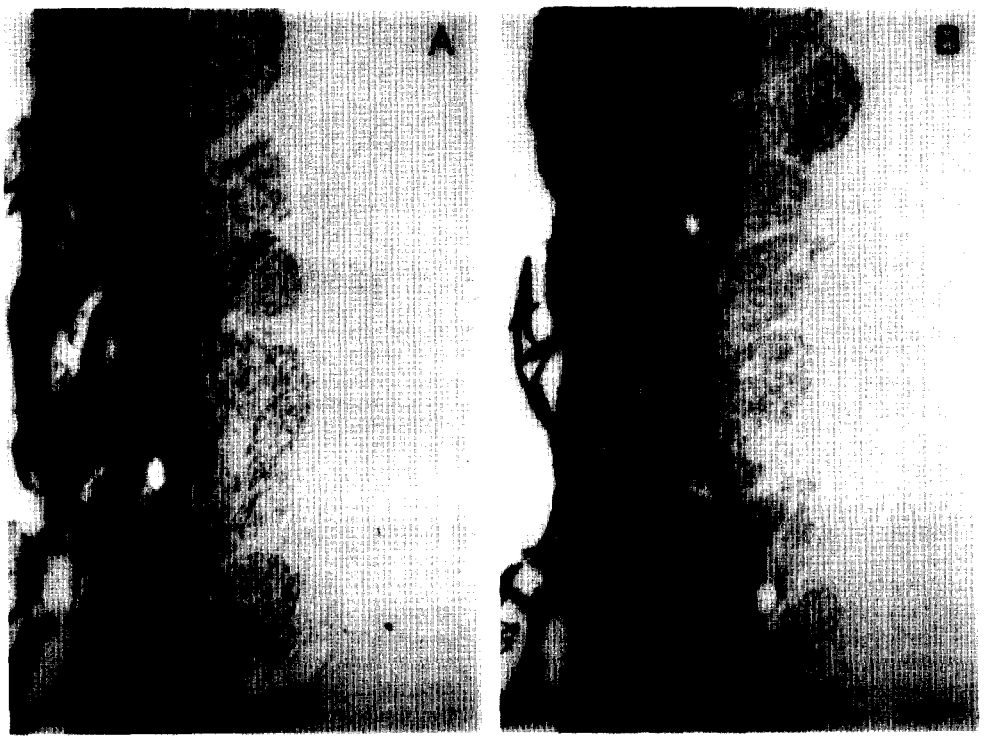

FIG. 4. Olfactory marker protein (OMP) immunoreactivity in 6- and 29-month-old F344 rats. OMP levels do not appear to decrease in aged as compared to young rats. Bar = 60 μm.

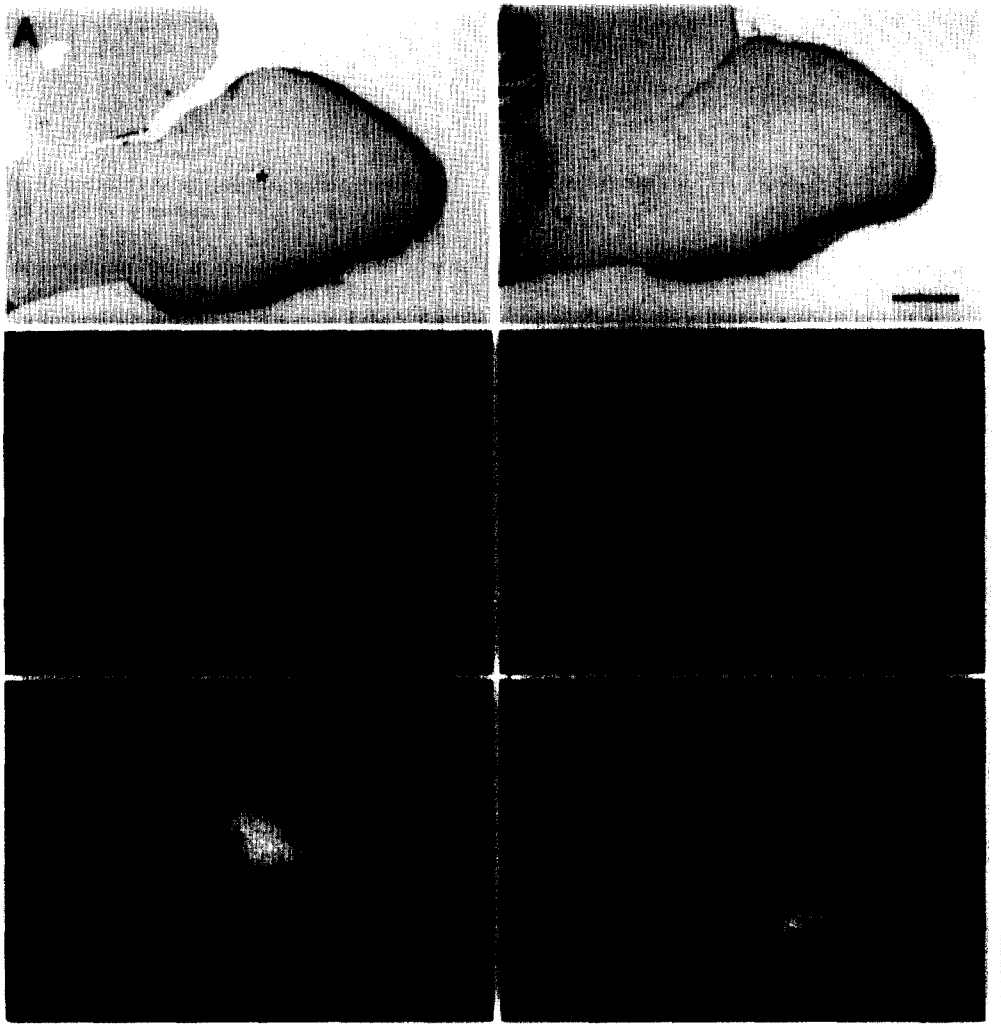

FIG. 5. Tyrosine hydroxylase immunoreactivity (A and B) mRNA (C and D) and glutamic acid decarboxylase (GAD) mRNA (E and F) in 6 (A,C,E)- and 20 (B,D,F)-month old C57Bl/6NNIA mice. Consistent with the lack of changes in the biochemical parameters, these photomicrographs illustrate the similarity in staining between young and aged mice. The asterisks indicate needle holes used to distinguish young and old animals that were processed simultaneously for comparative purposes. Bar = 300 μm.

zard, personal communication) suggests that at a comparable physiological age, that is at a time when age-related declines in health occur, the F1 hybrids might also show altered TH expression. Restricted diets delay but do not prevent the appearance of the age-related deficits in the F344 rats (86). The lack of change in TH expression in the two strains of mice argues against differential aging as the only contributing factor to the rat strain variations. Both mouse strains are relatively short-lived and it is difficult to obtain significant numbers of animals older than about 28 months. In fact, some of the mice obtained at 25 months died within a few days of arrival at the Burke facility, even before they could be analyzed. Even though the mice also exhibited many tumors and other age-related syndromes (e.g., alopecia and enlarged seminal vesicles), there were no age-related changes in olfactory bulb catecholamine expression. Analysis of older F344 Brown Norway F1 hybrids could indicate if the lack of alteration in TH expression in the hybrid rats is a reflection of chronological versus physiological age produced by their extended life-span.

A third factor is animal husbandry. In the Burke animal facility, all rats and mice were maintained in filter-topped cages in a laminar flow system which prevented access of infectious agents to, and transfer between, animals. The animals that did not show a decrease in TH expression were maintained in the Charles River facility, whereas the F344 animals were obtained from Harlan Sprague–Dawley. The role of differential husbandry remains to be explored, but as delineated next, there was no obvious indication of diseases in the olfactory system.

Previous studies in young animals demonstrated reciprocal relationships between the olfactory epithelium and bulb controlling both the development and maintenance of normal phenotypic expression (14,17,18,19, 22,26,44,45,46,49,52,53,54,57). Bulbectomy, which removes their targets, induces the death of mature olfactory receptor neurons (26,81). Similarly, both sensory deprivation and peripheral afferent denervation profoundly reduce expression of the dopamine phenotype in neurons intrinsic to the olfactory bulb (2,3,5,6,7,8,15,24,41,61,72,73). Following deafferentation, TH activity, immunoreactivity and mRNA

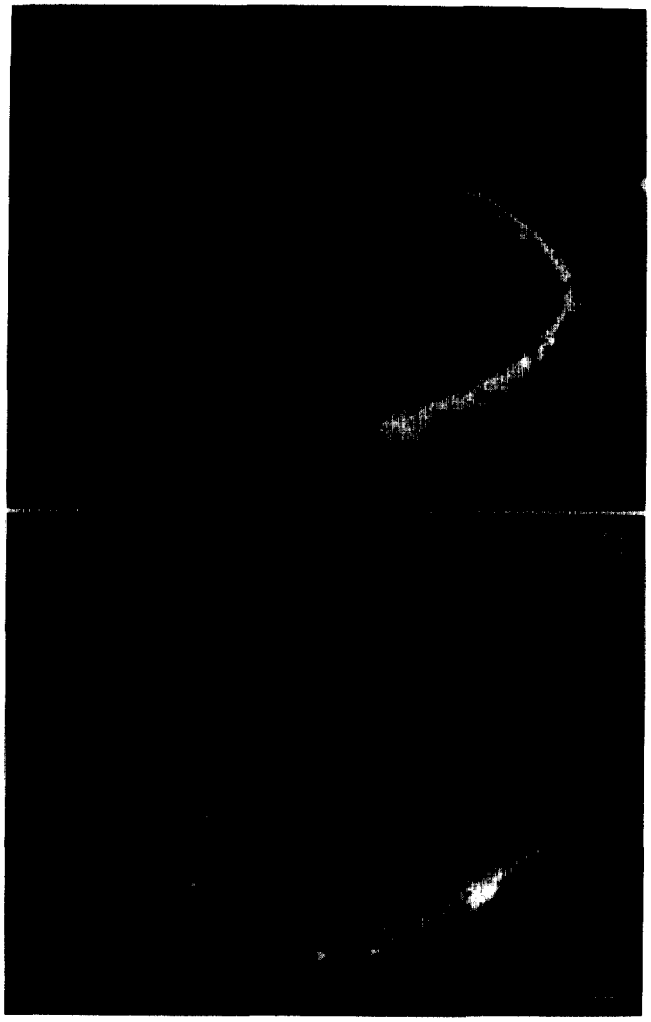

FIG. 6. Olfactory marker protein (OMP) message is found in the olfactory nerve layer of both young (A, 6-month) and old (B, 25-month) C57Bl/6NNIA mice. Bar = 500 μm.

levels all decline dramatically. The data suggest that expression of the dopamine phenotype is dependent on the integrity of peripheral afferent innervation. TH expression, for the most part, reflects the intrinsic dopaminergic system. Norepinephrine content, limited to centrifugal afferent innervation from the locus ceruleus, is maintained at normal levels in animals in which the dopamine content is reduced to 95% of control levels (5,6,61).

Because deafferentation alters TH expression, it was important to assess the integrity of afferent innervation. The F344 rats exhibited no apparent respiratory or nasal infection, a potential mechanism for destruction of the olfactory epithelium resulting in deafferentation. Expression of OMP, a protein found primarily in the cell bodies and processes (both dendritic and axonal) of mature receptor neurons also was assessed as an indication of the maintenance of peripheral afferent innervation (4). Both biochemical and immunohistochemical analysis of OMP levels demonstrated that afferent innervation was maintained at levels similar to those observed in young animals, that is, OMP levels remained constant across age. Interestingly, OMP mRNA could also be demonstrated in the olfactory bulb using in situ hybridization techniques. The presence of the message in olfactory axons in both rats and mice is in agreement with a previous report in the olfactory system (24,38,72,73) and is consistent with findings for TH message in other brain regions such as hypothalamic-pituitary dopamine systems (66).

TH expression in other brain regions of aged animals, such as the *substantia nigra* and *locus ceruleus*, of the F344 rat are consistent with those reported by other investigators (although there is much variability in the literature, see 11,27,39,50,51, 55,56,63,65,67,77,80,82,85, and 60 for review). In the present study, the dopaminergic system of the *substantia nigra* showed a large decrease in TH activity with no significant change in the *locus ceruleus*. Also in agreement with previous studies, TH activity in the adrenal medulla increased with age (39,42,63, 74,82,85). In some F344 rats there were obvious, often unilateral, tumors. On histological examination (manuscript in preparation and ref. 83) the tumors appeared to be adrenal medullary in origin and expressed high levels of TH and dopamine beta hydroxylase but not phenylethanolamine *N*-methyl transferase. For this reason, the analysis included only adrenal glands exhibiting protein concentrations similar to those observed in young and middle-aged rats. Even using these criteria a significant increase in TH activity occurred in the adrenal medulla in the same group of animals which exhibited a decrease in TH activity in both the olfactory bulb and *substantia nigra*, suggesting that in both the central and peripheral nervous system regulation of catecholaminergic expression is region specific. Numerous precedents exist for region and cell-type specific regulation of catecholaminergic systems of the CNS in aging animals (see above). Altered upregulation of TH in small groups of cells could occur before the formation of overt tumors. Preliminary histological analysis of adrenal glands that fell within the normal size-range did not indicate the presence of such pretumorous lesions (T. Wessel, personal communication).

The data presented indicate that, for the most part, TH expression in the olfactory bulb is relatively stable during aging. Changes in other neurotransmitters or receptors have not been examined extensively in the olfactory system. Those that have do not appear to change (see, e.g., 32). The relative lack of change in neurotransmitter expression in the olfactory bulb may reflect the necessity for this brain area to respond to continuous denervation and reinnervation in response to insult to the olfactory epithelium, and is consistent with the experiments demonstrating long-lived receptors in the olfactory epithelium (33,47,48) as well as continued neurogenesis in the adult olfactory bulb (12). The maintenance of the integrity of the olfactory system is consistent with the importance of olfactory function in these rodent species for behaviors such as reproductive, social, and ingestive functions. However, the question remains, do both the peripheral and central olfactory system exhibit the same degree of plasticity in young and aged animals? Diminished plasticity in aged animals has already been reported in the sympathoadrenal system (79). The maintenance of normal dopamine expression in aged mice and some rat strains permits future experiments to determine the effects of perturbation to the afferent innervation on the plasticity of response in the dopaminergic system. Studies are currently in progress to address this issue.

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