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The postnatal development of the main olfactory bulb of the rat

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Abstract. The postnatal development from birth to 1 year of the main olfactory bulb was examined quantitatively. The volume of the main olfactory bulb increased over seven-fold by day 30 and remained unchanged thereafter. During the same period the volume of the granular layer increased 18-fold and the mean areas of the olfactory glomeruli increased seven-fold. The mean areas of mitral cell perikarya doubled between the neonatal and juvenile periods. The total number of the mitral cells, however, declined during the first three postnatal weeks.

In the internal granular layer of the main olfactory bulb, 89% of the granule cells were acquired postnatally. Much of the cellular gain occurred during the first 3 weeks, with the period of maximum acquisition between days 8 and 14. The number of subependymal cells, the precursors of granule cells, reached a peak at 12 days and gradually declined. But some primitive cells could still be found at one year of age and there was an increase in the total number of granule cells beyond day 30. The mean number of internal granular layer cells in a single main olfactory bulb of adult rats was about 5×10^6; the number of mitral cells about 4×10^4.

In the animals injected with ^{3}H-thymidine on day 20 and killed 2 h after injection a small but significant proportion of cells was labelled in the subependymal layer but few in the internal granular layer. In the animals killed 20 and 40 days after injection there was a 10–11-fold rise in the proportion of labelled internal granular layer cells. The proportion of labelled internal granular layer cells decreased in longer survival groups but the total number of labelled cells remained the same, even in year-old animals. However, the total number of internal granular layer granule cells in the sections examined increased with age.

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Introduction

The main olfactory bulb is first recognizable in the rat on day 17 of gestation (day 0 being the first day of the detection of sperm) and by day 21 it has achieved its distinctive laminar organization (unpublished observations). Mitral cells form before birth and by the time of birth (day 22) they show some degree of maturity in terms of primary and secondary dendritic development (Scheibel & Scheibel, 1975) and their cell bodies are well-differentiated. The relatively early origin of mitral cells is in sharp contrast to the delayed acquisition of granule cells. ^{3}H-thymidine autoradiography revealed that many of the granule cells in the rat olfactory bulb form postnatally (Altman & Das, 1966; Altman, 1966, 1969) by migrating from the proliferative subependymal layer of the anterior horn of the lateral ventricle and olfactory ventricle into the internal granular, mitral cell, and glomerular layers.

Two types of experiment on the postnatal growth of the rat olfactory bulb have been performed. The first experiment was to establish the time course of granule cell acquisition, together with other parameters of olfactory bulb development. The second experiment investigated the question of whether the persisting production of granule cells after the time of weaning represents cell turnover or continuing cell acquisition.

Experimental

Experiment 1: growth of the olfactory bulb and some of its components

The following quantitative determinations were made in rats ranging in age from 0 to 360 days: volumetric growth of the olfactory bulb and of the granular layer; changes in the estimated number of subependymal cells and cumulative increases in the number of granule cells; changes in the areas of sections through the perikarya of mitral cells and through the glomeruli.

Animals. Two litters of Purdue-Wistar rats, culled to sixs males, were killed at each of the following postnatal ages: 0,1,2,3,4,5,6,8,10,12,14,16,18,20,25,30, and 90 days. Four additional males were killed at one year of age. Rats up to 8 days of age were killed by ether inhalation, older rats by Nembutal (6.5 mg/100 g body weight) followed by transcardiac perfusion with 10% (w/v) formalin (pH 7.0). The brains were removed, fixed in Bouin's solution (25% formalin, 6% acetic acid, 1% picric acid) for 24 h followed by repeated changes of 10% (w/v) formalin (pH 7.0), dehydrated in alcohols, and embedded in Paraplast.

Histology. Four brains from each group were randomly selected from this collection of normal material. Serial sections at a thickness of 10 μm were cut in either the coronal, sagittal, or horizontal plane through one or both of the olfactory bulbs of each animal. All sections were saved in animals less than 10 days of age, while one out of every four or two out of every four consecutive

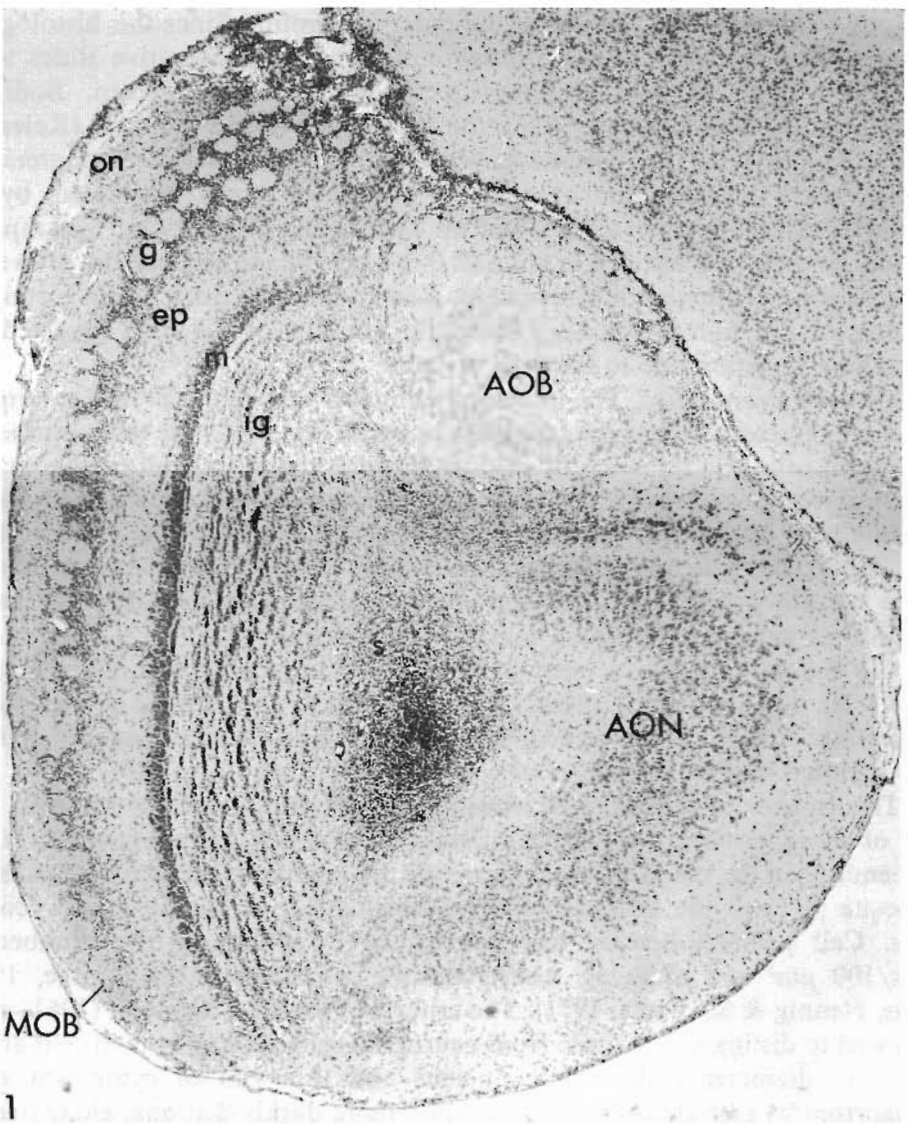

Figure 1. Coronal section through the olfactory bulb of a 10-day-old rat. The section, which was stained with haematoxylin and eosin, includes portions of the accessory bulb (AOB), the anterior olfactory nucleus (AON), and the various layers of the main olfactory bulb (MOB): the olfactory nerve fibre (on), the glomerular (g), the external plexiform (ep), the mitral cell (m), internal granular (ig), and the subependymal (s) layers. Linear magnification is ×25.5.

sections were discarded in most of the older age groups. Since this histological material was prepared to serve numerous purposes, consecutive slides were stained by one of four different methods: hematoxylin-eosin, Bodian's protargol-S-method (Bodian, 1936), the Weigert-Loyez myelin stain (Kelemen & Becus, 1969) or the Weigert-Loyez stain with cresyl violet counterstain (unpublished modification of the Weigert-Loyez stain developed by S. Evander). Measurements were taken on each of the olfactory bulbs sampling one out of every ten sections in animals 0–10 days of age and one out of twenty in those older. Although measurements were performed using sections stained by any of the four methods, Chi-square analysis of the data revealed no systematic variation due to the type of stain.

Parameters evaluated. The following estimates were made: (1) the volume of the main olfactory bulb and its component parts (Fig. 1); (2) the total number of the mitral cells and the areas of sections through the mitral cell perikarya; (3) areas of sections through the olfactory glomeruli; (4) the total number of granule cells in the internal granular layer, the diameter of the granule cells, and their packing density; (5) the total number of subependymal cells within the main olfactory bulb; (6) the granule to mitral cell ratio. The following procedures were used to obtain the above estimates.

Cell counts. Cell counts were made under oil immersion at a magnification of $\times 1500$. The number of mitral cells with nucleoli was counted at four points equidistant from each other along the mitral cell layer. A total length of 400 μm was sampled in each section.

The number of granule cells within an ocular grid superimposed upon 100 μm^2 of tissue was counted in four different areas of the internal granular layer. Depending on the plane of section, granule cell counts were taken from areas at opposite poles of the anterior-posterior, medial-lateral and/or dorsal-ventral axes. Cell packing density was determined by averaging the number of cells/100 μm^2 and applying Abercrombie's correction (Abercrombie, 1946; Elias, Hennig & Schwartz, 1971). The criteria of Smart & Leblond (1961) were followed to distinguish granule from neuroglial cells. Round or oval cells about 6 μm in diameter with a pale nucleus and thin rim of cytoplasm were categorized as granule cells, while smaller, more darkly-staining, elongated or irregularly-shaped cells were considered to be glia. Errors in distinguishing cells in the olfactory bulb are minimized by the physical separation of clusters of granule cells from the neuroglia within the fibre tract bundles.

Cell counts were taken in the subependymal zone of the main olfactory bulb in 100 μm^2 section.

Linear measurements. All the sections sampled were projected at a magnification of $\times 60$ with a Zeiss micro-projector and traced on paper. The length of the mitral cell layer tracing was determined with a map reader to the nearest 0.1 cm. The diameters of twenty granule cells with circular outlines from

two sections in each animal were measured using a Vicker's image-splitting eyepiece to the nearest 0.01 μm. Subependymal cell diameters were similarly measured, as well as the nucleolar diameters of twenty mitral cells in a 0, 20, and 360-day-old brain, each coronally sectioned.

Measurements of area. All planimetric measures were taken to the nearest μm^2. The outline of the main olfactory bulb and the subependymal zone were traced at a magnification of $\times 60$. The total areas of the main olfactory bulb, the subependymal zone and the internal granular layer were determined by planimetry. The projected outlines of twenty mitral cells, anterior or medial in position, were traced from each bulb at a magnification of $\times 667$, and the areas calculated. Cells that appeared to be sectioned in a plane parallel to their vertical axes and had clearly visible nucleoli were used.

Olfactory glomeruli in two sections per animal were magnified $\times 300$ and traced on paper. The outlines of the ten largest glomeruli per section were selected for measurement of area.

Transformations of the data. Numbers of granule and subependymal cells per section were calculated by summing the products of the cell densities and the corresponding areas. The total number of mitral cells per section was calculated by multiplying the number of mitral cells per unit length by total length of the mitral cell layer.

The volume of the olfactory bulb, as well as the total number of mitral, granule and subependymal cells were determined using the techniques of Smith (1934b) and Dornfeld, Slater & Scheffé (1942). The number of cells within each section and the area of each section were plotted for the individual animals. Curves were drawn between the points and the area beneath the curves planimetrically measured. The total volume was calculated with the following formula: $V = XYPt$, where $V =$ vol. in mm^3; $X =$ number of sections/mm on the graph; $P =$ the planimetric reading in mm^2 and $t =$ the thickness of the sections in mm. The total volume of the main olfactory bulb was subdivided as follows: (1) the volume of the layers external to an including the mitral cell layer; (2) the volume of the internal granular layer; (3) the volume of the subependymal zone.

Cell numbers were calculated in a similar manner to the total volume. Abercrombie's correction was applied to offset the number of segments of cells included in the sample counts.

Statistical analysis. A model 1 one-way analysis of variance to test for an overall age-effect was performed on most of the parameters measured. When the age effect was significant, individual comparison tests between group means (Newman-Keuls) were calculated. Tests for homogeneity of variance (Cochran's C and Bartlett's Box F) were also performed (Winer, 1962). In the cases where the variance was not homogeneous, the Kruskal-Wallis one-way analysis of variance and randomization tests for two independent samples were the statistical methods used (Siegel, 1956).

Experiment 2: fate of the late-forming granule cells

The fate of the granule cells formed after the end of the suckling period (Altman, 1969) was investigated autoradiographically in rats that were injected with ^{3}H-thymidine on day 20 and killed at various intervals thereafter.

Animals. Twenty-four male Purdue–Long-Evans rats were injected with 10μCi/g body weight of ^{3}H-thymidine on day 20 and were divided into groups of 4 animals with the following survival periods: 2 h, 20, 40, 70, 160 and 340 days. A split-litter design was used: an animal from each litter was assigned to one of the survival periods. Animals were killed and their brains embedded in paraffin as previously described for Experiment 1.

Histology. Coronal sections of the olfactory bulbs were cut at a thickness of 6 μm, one out of every 10 sections saved. Slides from three different levels of the olfactory bulb were selected for autoradiographic analysis: (1) a rostral section including just the tip of the internal granular layer; (2) a posterior section including the most rostral portion of the accessory olfactory bulb; (3) and an intermediate section. One slide of the subependymal zone bordering the anterior horn of the lateral ventricle was also selected. These slides were coated with a nuclear emulsion, exposed in light-tight boxes for 90 days and developed with Kodak D–19. The slides were post-stained with hematoxylin-eosin and coverslipped.

Parameters evaluated. Cell counts were made under oil immersion at a magnification of $\times$1500 with an ocular grid superimposed over 100 μm^2 of tissue. Eight grid samples per section were taken from the internal granular layer in the main olfactory bulbs. One grid sample of the subependymal zone was counted in each appropriate section. Cells were categorized as labelled or unlabelled, and the percentage of labelled cells calculated. A cell was considered labelled if it had five or more silver grains lying over the nucleus.

In addition, the sections were projected at a magnification of $\times$65 with a microprojector and traced on paper. The areas of the internal granular layer as well as the total number of granule cells and labelled cells in these sections were calculated.

Results

Experiment 1

Differences due to the plane of sectioning. To ascertain whether the measurement of area of diameter varied with the plane of section, the largest means in each group for the various measures were identified and categorized according to their plane of section. Chi-square tests revealed that neither subependymal nor granule cell diameters, nor glomerular areas varied with the

plane of section. With the exception of the migrating cells, all must assume a spherical shape. The areas of the projected outlines of the mitral cell perikarya, however, were consistently larger in those brains sectioned horizontally.

Volume of the main olfactory bulb. Volumetric changes of the main olfactory bulb and its layers are illustrated in Fig. 2. The growth curve of the entire bulb is characterized by a steady moderate rise in volume during the first post natal week, a more rapid rise for several days during the second week, followed by a third phase of very gradual growth. Within-group variability was low during the first postnatal week but increased thereafter; sample population variances were not homogeneous. The Kruskal-Wallis one-way analysis of variance revealed a significant increase with age in total volume of the main olfactory bulb ($H=281.22$, d.f. = 71, $P<0.001$). There were significant differences between the following age groups (randomization test, one-tailed, $P<0.025$); (1) 0–1 days and all other groups; (2) day 2 and groups 4 days and over; (3) day 3 and groups 5 days and over; (4) 4–5 days and groups 6 days and older; (5) 6–8 days and groups 10 days and older; (6) day 10 and days 20 through 30; (7) 12–20 days and day 30.

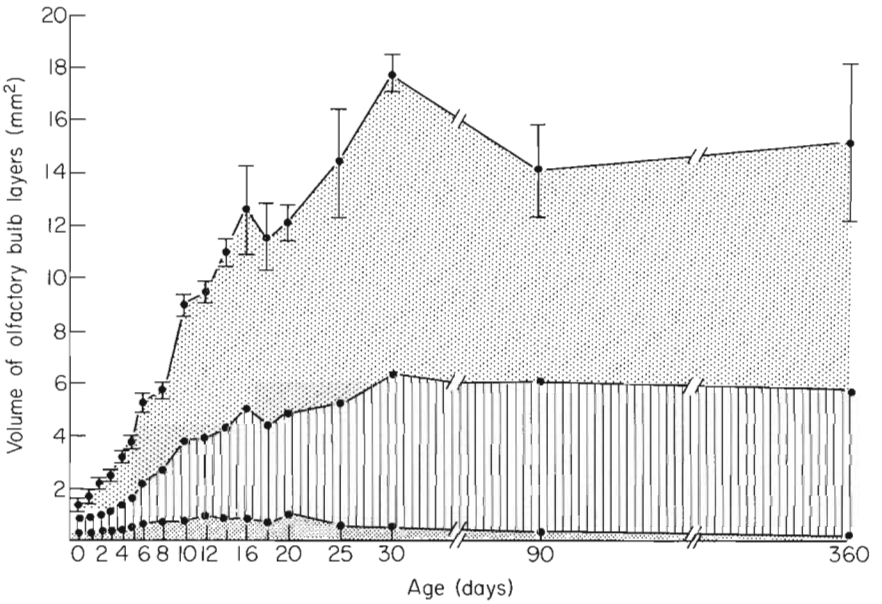

Figure 2. Volumetric growth of the main olfactory bulb and of the layers external to and including the mitral cell layer, the internal granular layer and the subependymal zone. The upper line refers to the volume of the main olfactory bulb, the vertical bars are equivalent to 2 SEM. ⁞⁞⁞, volume of layers external to and including mitral cell layer; (||||), volume of internal granular layer; ▒, volume of subependymal zone Kruskal-Wallis one-way analysis of variance on total bulb volume: $H=281.22$, d.f. = 71, $P<0.001$.

The volume of the layers external to and including the mitral cell layer increased with age ($H=281.25$, d.f. = 71, $P<0.001$). Significant differences (randomization test, one-tailed, $P<0.025$) were found between the following groups: (1) day 0 and all other groups: (2) 1–3 days and groups 4 days and older; (3) 4–5 days and groups 6 days and older; (4) day 5 and days 10–30; (5) day 8 and groups 10 days and older; (6) day 10 and days 25–30; (7) days 12–14 and day 30.

The volume of the internal granular layer increased with age ($H=283.09$, d.f. = 71, $P<0.001$) and the following mean differences were significant (randomization test, one-tailed, $P<0.025$): (1) day 0 and groups 2 days and older; (2) day 1 and groups 3 days and older; (3) days 2–3 and groups 3 days and older; (4) days 4–6 and groups 8 days and older; (5) day 8 and groups 10 days and older; (6) day 10 and days 16, 20, 30 and 360; (7) days 12–14 and days 20 and 30; (8) days 16–25 and day 30.

The volume of the subependymal layer also varied with age ($H=261.21$, d.f. = 71, $P<0.001$). The groups which differed from one another (randomization test, two-tailed, $P<0.05$) were as follows: (1) days 0 and 1 had smaller subependymal volumes than days 5–20; (2) day 2 than days 5–12 and 16–20; (3) days 3 and 4 than days 8–12 and day 20; (4) day 5 than days 10–12 and day 20; (5) day 360 and days 10–12.

Mitral cell measurements. The analysis of variance on the number of mitral cells (Fig. 3A) indicated an age effect ($F=5.26$, d.f. = 17/54, $P<0.001$). Within-group variance was high for all age groups, but was homogenous. The following comparisons were significant (Newman-Keuls, $P<0.05$): (1) day 90 animals had fewer mitral cells than days 2–8 and days 14; (2) days 20–360 and days 0 and 1 had fewer cells than days 6 and 10.

Because there appeared to be a gradual decline of mitral cells (Fig. 3a) from the third week of life, a preliminary trend analysis was performed on our original data. The analysis of variance and subsequent regression analysis (Winer, 1962) indicated a linear decline in the number of mitral cells from the first postnatal week to day 20.

To verify this apparent decline in mitral cell number in early postnatal life, a second set of twelve males from two litters was collected at postnatal ages 0–6,8,10,12,14,16,18 and 20 days. Four brains from each group were randomly selected and the number of mitral cells in the right olfactory bulb was estimated for each animal. The procedures differed from those already described in the methods section in the following ways: (1) all brains were sectioned in the sagittal plane and stained with hematoxylin-eosin; (2) each section was coded so that the person collecting the data was unaware of the animals' age; and (3) 1 out of every 10 sections was sampled from each brain.

The analysis of variance on this second collection of data was also significant ($F=2.07$, d.f. = 13/42, $P<0.05$). The analysis of variance performed

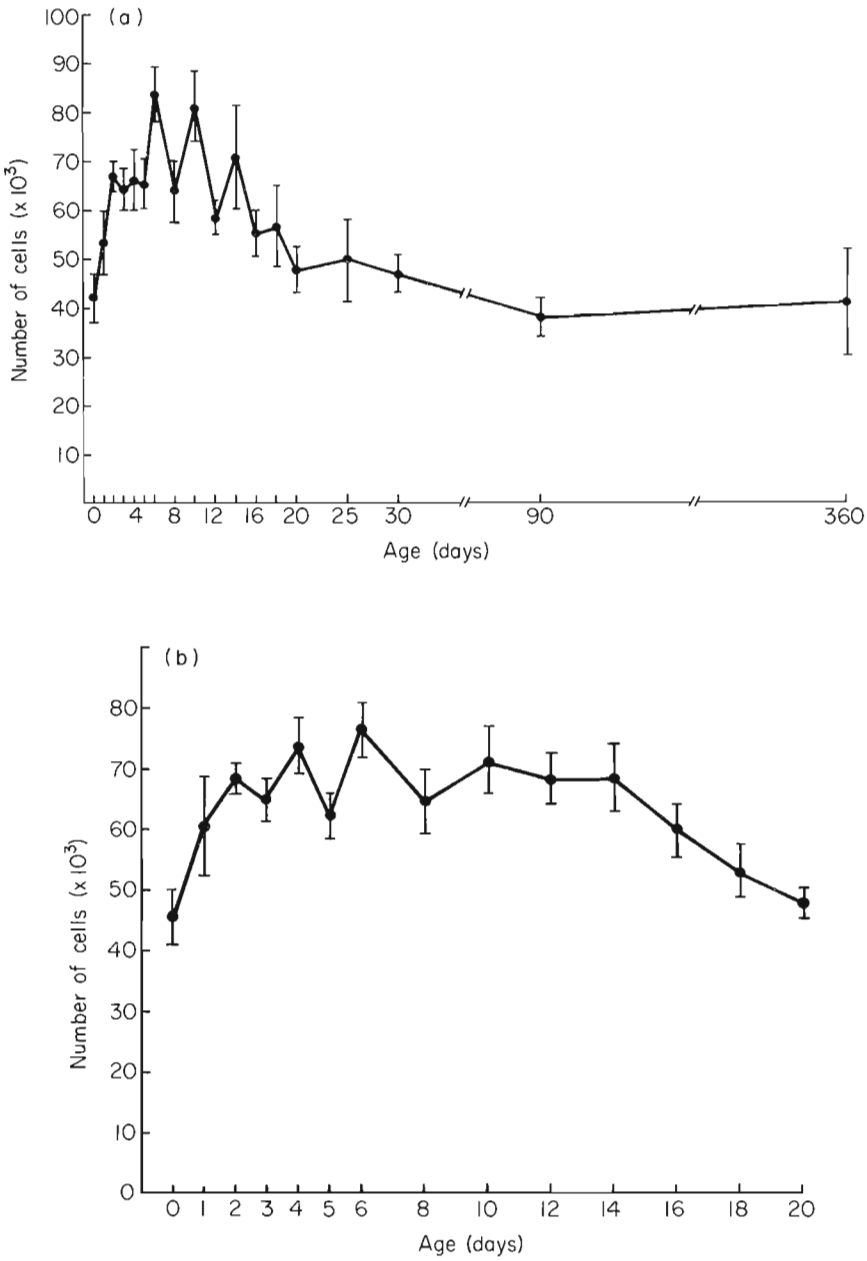

Figure 3. Age changes in the estimated number of mitral cells in the main olfactory bulb (a) data from rats of 0–360 postnatal days. Analysis of variance: $F(17,54) = 5.26$, $P<0.001$. (b) Pooled data from original and replicated experiments showing a decline in the number of mitral cells by the age of weaning. Analysis of variance: $F(13,28) = 2.74$, $P<0.025$. Vertical bars represent 2 SEM.

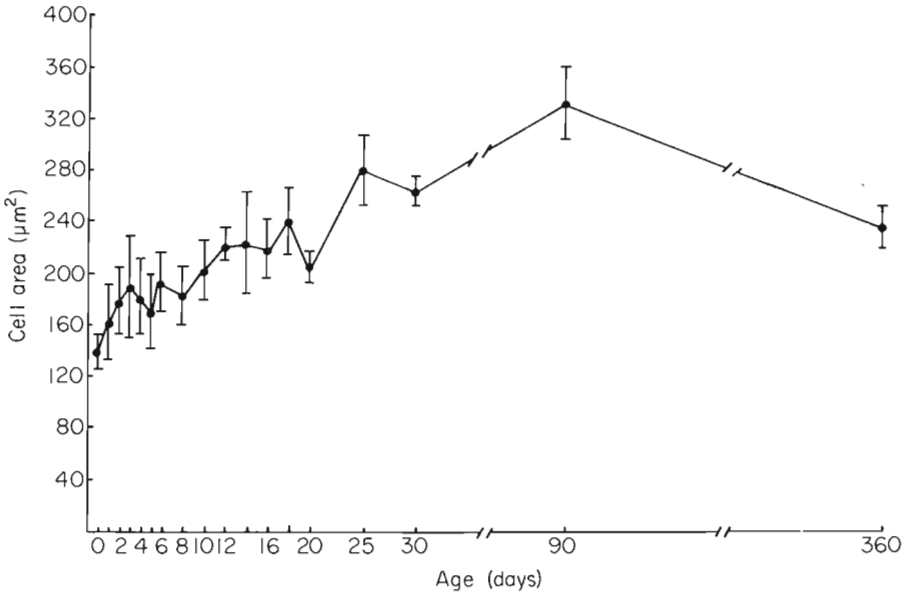

Figure 4. Age changes in the mean areas of the projected outlines of mitral cell perikarya. Vertical bars represent 2 SEM. Analysis of variance: $F(17,54) = 2.52$, $P<0.01$. Newman–Keuls: $P<0.05$, 0/25, 0/90.

on all groups differing in age by an interval of 2 days was significant ($F=3.76$, d.f. = 10/33, $P<0.01$) and the subsequent test for linear trend (Winer, 1962) was significant ($F=4.38$, d.f. = 1/33, $P<0.05$).

The two separate samples did not significantly differ from one another ($F=0.10$, d.f. = 1/97, $P>0.10$) and the age effect was significant when the data was simply pooled (Fig. 3b, $F=3.80$, d.f. = 13/98, $P<0.01$) or when procedures for replicated experiments (Winer, 1962) were used ($F=2.74$, d.f. = 13/28, $P<0.025$). The following comparisons were significant (Newman–Keuls, $P<0.05$): (1) day 0 animals had fewer cells than days 2–6 and days 10–14; (2) day 20 rats had fewer cells than days 4, 6 and 10. The evidence indicates that there is a genuine decline in the number of mitral cells in the mitral cell layer beginning approximately at the potential age of weaning.

There was a significant effect across age groups in the area of the projected outlines of mitral cell perikarya ($F=2.52$, d.f. = 17/54, $P<0.01$). The mitral cells in the young juvenile and adult animal are twice the size of those in the newborn (Fig. 4). The mean perikaryal area is 140 sq μm in the neonate, compared to 280 sq μm in the 25-day age group. Differences in the means

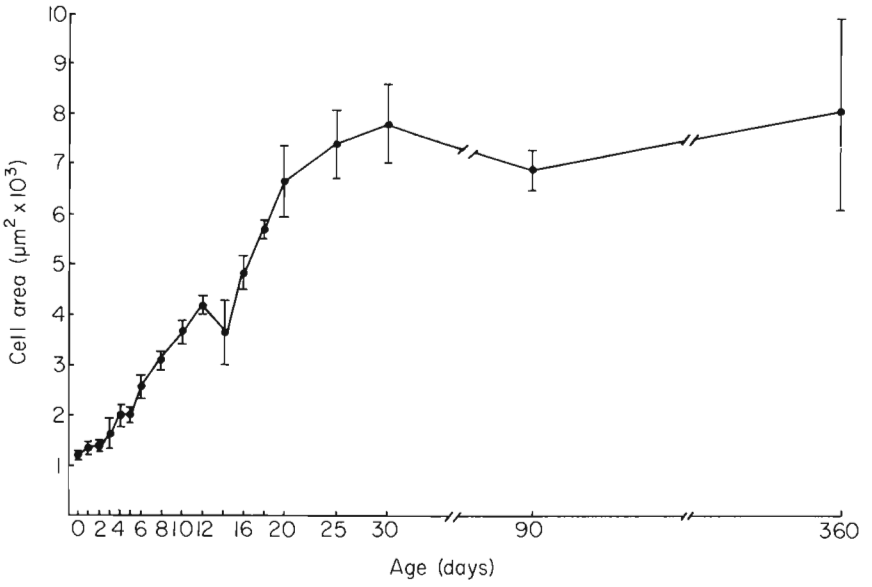

Figure 5. Age changes in the mean area of the olfactory glomerulus. Vertical bars represent 2 SEM. Kruskal–Wallis one-way analysis of variance: $H = 282.52$, d.f. = 71, $P < 0.001$.

between the 0 and the 25- and 90-day groups were significant (Newman–Keuls, $P < 0.05$). The actual differences are no doubt even greater than indicated here, since, at the earliest ages, the cells selected to be traced and measured were biased in favour of those that were among the largest and most easily identified.

Mean diameters of nucleoli in coronal sections of the 0, 20 and 360-days-old animals were 2.15, 2.80 and 2.99 μm, respectively. The diameter of the mitral cell nucleolus was significantly larger in the 20 or 360-days-old animals than at birth ($t = 2.10$, d.f. = 19; $t = 2.69$, d.f. = 19, $P < 0.05$, one-tailed test). There was no difference in nucleolar diameter between the 20 and 360-days-old animals.

Glomerular area. Figure 5 illustrates nearly a seven-fold increase in mean glomerular area between the 0- and 360-day age groups, from approximately 1220 to 7800 μm^2. Variance increased with age and was not homogeneous. The Kruskal–Wallis one-way analysis of variance showed a significant age effect ($H = 282.52$, d.f. = 71, $P < 0.001$) and the following between group comparisons were significant (randomization test, one-tailed, $P < 0.025$); (1) days 0–2 and 4 days and older; (2) days 3 and 5 and days 8–12 and 16–360; (3) day 4 and days 10–12 and 16–360; (4) day 6 and days 8–12 and 16–360; (5) day 8 and days 12 and 16–90; (6) day 10 and days 16–90; (7) days 12–14 and days 18–90; (8) day 16 and days 30–90; (9) day 18 and day 30.

Granule cell measurements. The increase in granule cells in the internal granular layer (Fig. 6) is similar to the volumetric growth of the main olfactory

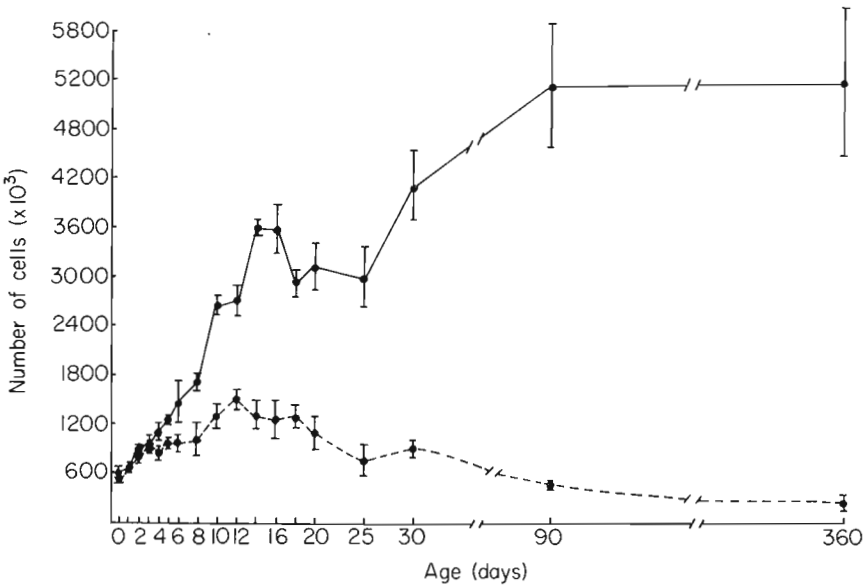

Figure 6. Age changes in the number of granule cells in the internal granular layer and the number of subependymal cells. Internal granular layer (–), subependymal layer (– – –). Vertical bars represent 2 SEM. Analysis of variance: $F(17,54) = 15.4$ and 5.1, respectively, $P < 0.001$.

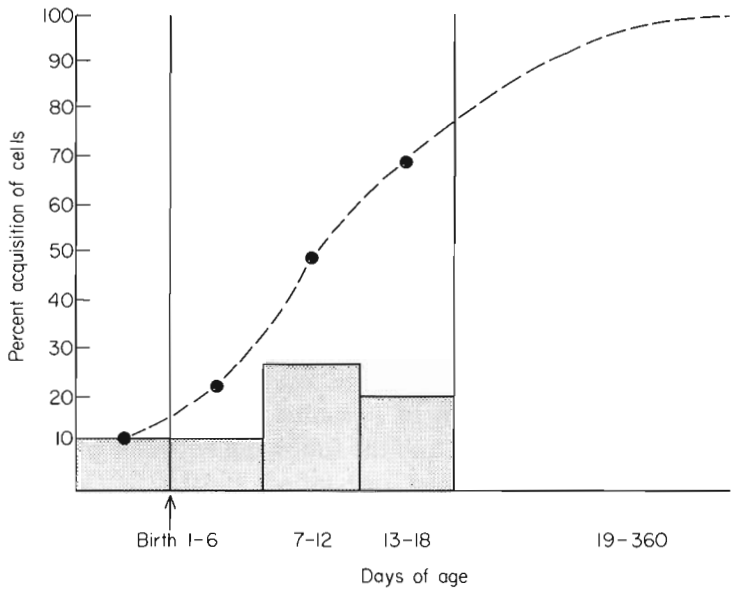

Figure 7. Percentage acquisition of granule cells during various age intervals and the cumulative percentage acquisition. 100% acquisition is based on the average number of cells in the 30, 90- and 360-day age groups. ⬚, percentage acquisition; (–●–), cumulative percentage acquisition.

bulb, with a sharp rise between 8 and 14 days and a gentle increment thereafter. The analysis of variance was significant at the $P<0.001$ level ($F=15.4$, d.f. = 17/54). The Newman–Keuls multiple range test revealed significant differences ($P<0.05$) in granule cell number between the following age group: (1) days 0 through 5 and 10 days and older; (2) day 6 and days 14, 16, 20, 30, 90 and 360; (3) day 8 and days 14, 16, 30, 90 and 360; (4) days 10–25 and days 90 and 360.

Because the variance in the older age groups was high, the mean number of granule cells in animals aged 30 to 360 days was designated as 100% acquisition. At birth (Fig. 7) 10% of the granule cells of the year old animal have been acquired, 20% by day 6, 45% by day 12 and 65% by day 20. The maximum percentage of cells is acquired during the 7–12-day period, approximately 4%/day compared to 1.5 and 3.0% during the first and third postnatal weeks, respectively. Later, the acquisition rate falls to between 0.1 and 0.2% per day.

An analysis of variance of granule-cell packing density in the internal granular layer proved to be significant across age groups ($F=15.83$, d.f. = 17/54, $P<0.001$). Packing density was high at birth and shortly after (110 cells/10^5 μm^3), decreased precipitously between 3 and 8 days, and was followed by a more gradual decline (to 75 cells/10^5 μm^3 by day 25). Packing density again rose somewhat between 30 and 360 days, reflecting either an artifact of shrinkage due to the fixation process, or an indication of the incorporation of more and more granule cells within a stable internal granular layer volume.

The diameters of the granule cell perikarya varied between 4.5 and 9 μm with no differences across age groups. Most cells were within a 5–7-μm range. The mean diameter used in Abercrombie's formula for estimating the corrected number of granule cells was 6.68 μm.

Subependymal cell measurements. The number of subependymal cells within the main olfactory bulb rose steadily, peaked at 12 days and decreased thereafter (Fig. 6). The age effect was significant at the $P<0.001$ level ($F=5.1$, d.f. = 17/54). There are significantly more cells at 12 days than on days 0 to 1 (Newman–Keuls, $P<0.05$). The 90- and 360-day age groups had fewer subependymal cells than all groups between the ages of 10 and 20 days, though they did not differ from each other. The 360-day group also had fewer cells than the 3-, 5–10- and 30-day groups.

Subependymal cell diameters did not vary with age and were usually in the range of 4–6 μm. The mean diameter used in Abercrombie's formula for estimating the corrected number of complete cells was 5.5 μm.

Granule to mitral cell ratios. The ratio of granule to mitral cell numbers follows an S-shaped growth curve (Fig. 8). At birth the ratio is 12.6 and at one year is 145.5. Variance increased dramatically with age and was not homogeneous. The Kruskal–Wallis one-way analysis of variance revealed a significant age effect ($H=265.78$, d.f. $=71$, $P<0.001$) and the randomization test (one-tailed, $P<0.025$) showed significant differences between the following

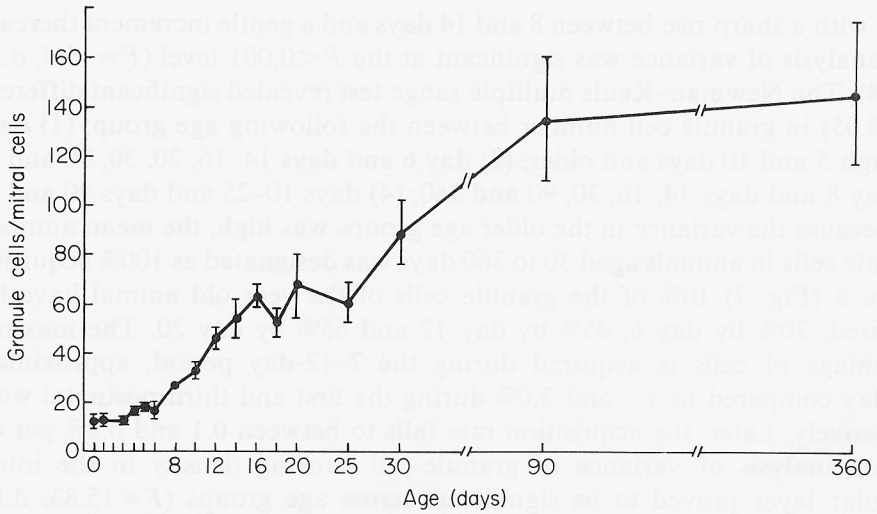

Figure 8. Age changes in the granule to mitral cell ratio. Vertical bars represent 2 SEM. Kruskal–Wallis one-way analysis of variance: $H=265.78$, d.f. = 71, $P<0.001$.

age groups: (1) day 0 and 1 and days 8–360; (2) day 2 and days 4, 5, and 8–360; (3) days 4–6 and days 8–360; (4) day 8 and days 12–360; (5) day 10 and days 12 and 16–360; (6) day 12 and days 30–360; (7) days 14–25 and days 90–360; (8) day 30 and day 90.

Experiment 2

Percentage of labelled cells. The labelling indices in the subependymal zone varied significantly with survival time ($F=8.9$, d.f. = 5/18, $P<0.001$). The 2 h survival group with a labelling index of 6.5% significantly differed from all other survival groups (Newman—Keuls, $P<0.05$) since the labelled cells had not had sufficient time to migrate from the germinative matrix to the internal granular layer. The average labelling index for the longer survival groups was 0.8%. These cells were assumed to be labelled non-migratory cells.

The analysis of variance of the percentage of labelled granule cells in the internal granular layer of the main olfactory bulb was significant ($F=19.65$, d.f. = 5/18, $P<0.001$). The 2 h survival group had a significantly smaller percentage of labelled cells in the internal granular layer than all other groups with the exception of the 360-day group (Newman–Keuls, $P<0.05$). The 20- and 40-day survival groups had a 4.9 and a 5.8% labelling index respectively. These were significantly different than the 70-, 160- and 340-day survival groups which had labelling indexes of 2.7%, 3.2% and 2.6% (Fig. 9). An analysis of variance performed on the total number of labelled granule cells as a function of survival time was non-significant ($F=1.80$, d.f. = 4/15, $P>0.05$). (The 2-h survival group was omitted from this analysis.)

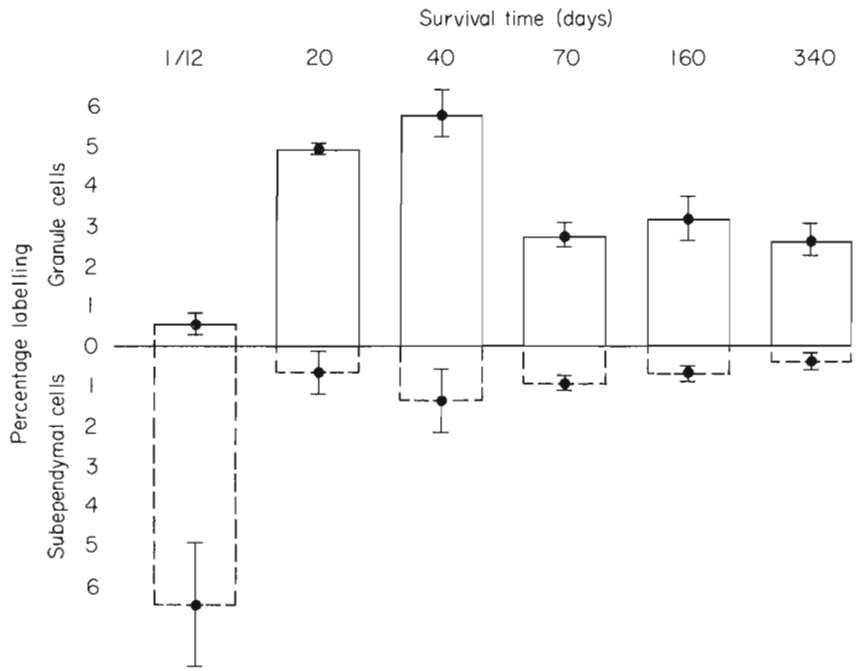

Figure 9. Percentage of labelled granule and subependymal cells as a function of survival time. Vertical bars represent 2 SEM. Analysis of variance: $F(5.18) = 19.65$ and 8.9, respectively, $P<0.001$.

The total number of granule cells in the internal granular layer in the selected sections significantly increased with age ($F=11.84$, d.f. = 4/15, $P<0.001$). Mean comparisons indicated that the 40-day group had significantly fewer cells in the selected sections than did the 90-, 180- and 360-day groups (Newman–Keuls, $P<0.05$). In addition, the 60-day group had significantly fewer cells than the 180- and 360-day groups.

Discussion

Volumetric changes

Our data indicate that the total volume of the main olfactory bulb reaches an asymptote about 30 days of age. Smith (1934b), also studying a Wistar strain, found that the volume of the fixed main olfactory bulb undergoes a 23% increment between day 28 and 1 year. A recent study of Sprague–Dawley rats (Hinds & McNelly, 1977), also found increases in olfactory bulb volume

between animals aged 3 months and 1 year. Our negative findings in terms of volumetric growth after day 30 may reflect strain differences or differences in the amount of tissue shrinkage as a result of fixation and histological procedures. However, cell number estimates remain unaffected by these factors and are more reliable measures of structural development.

Mitral cell number

Our study and the one by Hinds & McNelly (1977) derived similar estimates of mitral cell number in the adult rat, 40 000 compared to 45 000 cells. Neither study found a change in mitral cell number between 90 and 360 days. The rapid decline in mitral cell number that we found during the second and third postnatal weeks may reflect a loss of cells through cell death. This possibility is supported by the observation that many of the mitral cells in the early postnatal ages seemed to be undergoing degenerative changes. The apparent postnatal loss of mitral cells may be secondary to a lack of input from the olfactory epithelium or may indicate an overproduction of mitral cells with respect to the 'normal' complement of cells.

The lower number of mitral cells in the neonatal ages was an unexpected result. It may have been due to a difficulty in identifying immature mitral cells in a densely populated multicellular layer. At later developmental stages, however, the mitral cells are large and widely spaced within a monolayer.

Acquisition of granule cells

During the rapid phase of granule cell increase, roughly the first 2 weeks, there was an increase in the population of subependymal cells, the precursors of granule cells. Thereafter, the subependymal cell population declined and the increase in granule cell number decelerated. But both the cell counts in the normative material (Purdue–Wistar strain) and the labelling in the autoradiographic study (Purdue–Long-Evans strain) indicated prolonged acquisition of granule cells in the internal granular layer. After day 30 the increase in granule cells was modest and not significant in one study. However, the total number of granule cells in the autoradiographic material showed a significant net gain between the 60-day and the 180- and 360-day groups. The total number of labelled granule cells remained the same across age groups. In contrast to the loss in mitral cells, then, there was some gain in granule cells and a dramatic rise in the granule/mitral cell ratio during the period studied.

Within-group variability

Within-group variability increased with age. In groups less than a week old there was little variation in measures of main olfactory bulb volume, number of

differentiated granule cells, granule to mitral cell ratios or glomerular area. We suggest that differential exposures to viral or bacterial infections or airborne chemicals which affect the survival or activity of neurons in the olfactory epithelium during development could be responsible for the increased variability in olfactory bulb anatomy with age.

Functional considerations

Both the internal granular layer and the region designated as the 'external layers' contribute to the substantial growth of the olfactory bulb after birth. The growth in the volume of the internal granular layer is largely attributable to the increase in the number of granule cells, with some contribution made by the decrease in their packing density. Since the effect of the increase in the size of mitral cell perikarya was partially cancelled by the decrease in their number, one important contributing factor to the growth of the 'external layers' must have been the great increase in glomerular size. The rise in the granule/mitral cell ratio raises the question whether or not the proportional increase in the interneurons of the bulb with age is associated with improved olfactory function.

The spontaneous electrical activity characteristic of the olfactory bulb is evident by day 3 or 4 (Salas, Guzman-Flores & Shapiro, 1969). The average frequency of burst activity increases rapidly until day 12, but does not reach adult levels until day 24. Salas, Shapiro & Guzman-Flores (1970) also obtained evidence that differential mean burst frequencies to two odours become increasingly distinct after postnatal day 12. The electrophysiological evidence correlates well with the pattern of granule cell acquisition and differentiation within the olfactory bulb observed here, with 45% of the cells acquired by day 12. The parallel development of olfactory rhythmic potentials and granule cell acquisition is reconcilable with the notion that the dendrodendritic synaptic interactions between mitral and granule cells are largely responsible for this spontaneous activity (Shepherd, 1970).

The behavioural significance of delayed granule cell acquisition is not known. Behavioural observations indicate that olfactory cues play an important role in the behaviour of the rat from infancy. Welker (1964), for example, has reported that 2-day-old rats respond to odours by sniffing and changing their respiratory rates. Similarly, Schapiro & Salas (1970) noted an inhibition of motor activity in infant rats as early as 6 days in response to the odour of the mother. Pups will also display a preference for homecage shavings over clean wood-shavings or shavings from non-pregnant females by 7 days of age (Tobach, Rouger & Schneirla, 1967; Gregory & Pfaff, 1971) and, by day 14 are sensitive to the anal excreta of lactating females (Leon & Moltz, 1972). Rat pups bulbectomized during the first postnatal week exhibit little nursing behaviour

and soon die; sparing a caudal portion of the bulb reduces the effect (Singh & Tobach, 1975).

It is not clear whether one or both of the major olfactory systems is responsible for the early responsiveness to olfactory stimulation. The accessory olfactory bulb appears to mature earlier (Hinds, 1968), but the anatomical evidence suggests that even the main olfactory bulb may be functional to a limited extent in early postnatal life. Despite the small size of its components and few granule cells, glomeruli are distinct and at least some cells in all the representative categories appear to be well differentiated at birth (Scheibel & Scheibel, 1975). By this time, synapses have already formed in the olfactory cortex in the rat (Westrum, 1975) and, in the mouse (which has a shorter gestational period than the rat) these make their initial appearance on embryonic day 15 (Derer, Caviness & Sidman, 1975; Hinds, 1975).

If the main olfactory bulb does mediate early postnatal olfactory ability, however, granule cells, which are not only few in number but may not become synaptically mature for some time, must have little role in these functions. Supporting this hypothesis, we have found that eliminating migrating and immature granule cells through low-level X-ray-irradiation does not interfere with an infant's ablity to home to wood-shavings from the home cage (Yanai & Rosselli-Austin, 1978). Evidence is accumulating (Walters & Struble, 1978) that preventing the development of the postnatally-formed granule cells has no effect on body growth during the suckling period, but growth is retarded after weaning and abnormalities develop in food selection in adult rats. The availability of milk to suckling rats obviates the need for diet selection, and the uniformity of the nest places little demand on the appreciation of different odours—conceivably the granule cells become functionally significant after weaning when the animal encounters a more heterogeneous environment.

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