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SCIENCE

Neurons in the Rat Dentate Gyrus Granular Layer Substantially Increase During Juvenile and Adult Life

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Abstract. *Volumetric estimates of the total number of granule cells in rats 30, 120, 200, and 365 days old increase linearly by approximately 35 to 43 percent between 1 month and 1 year. Total volume of the granular layer also grows linearly during that time. These results demonstrate a numerical increase in a neuronal population during adulthood in the mammalian brain.*

Since 1963 (*1*) it has been repeatedly shown with [^{3}H]thymidine autoradiography that dentate granule cells in the hippocampus continue to be produced during the adult period in rats (*2, 3*). The question remained whether these neurons were adding to the population or were replacing those that may die during adult life. By 1975, Bayer and Altman (*4*) obtained circumstantial evidence that the number of granule cells was increasing until 120 days of age; the present study was designed to determine numerical age changes in this group of neurons.

We estimated the number of granule cells in the hippocampus of 17 male Purdue-Wistar rats, four animals each at 30, 120, and 200 days, and five at 365 days. After a transcardial perfusion with 10 percent neutral Formalin and Bouin's fixative, the brains were stored in 10 percent neutral Formalin until the block containing the entire right hippocampus was embedded in methacrylate. Serial 3-μm slices were cut in the horizontal plane with a JB-4 microtome (Sorvall) and stained with cresyl violet. A running count was made of all slices containing the granular layer; care was taken to assure that slices were of uniform thickness.

Two previous volumetric estimates of granule cell numbers in rats used modified Abercrombie correction factors to counts of neuronal nucleoli (*5, 6*). This method was unworkable in our preparations since the Purdue-Wistar strain consistently shows double nucleolus-like bodies in many granule cell nuclei (arrows in Fig. 1). Consequently, we chose to quantify the nucleus. Our method was based on the equation:

$$N = V_t/V_a$$

where N is the total number of cells, V_t the total volume of all cell nuclei, and V_a the average nuclear volume (*7*).

We experimentally determined V_t from low-magnification photomicrographs of dorsal and ventral (*6*) granular layer slices at regularly spaced intervals. The granular layer was divided into 25-μm wide strips running perpendicular to the length; each strip was a possible sample. We randomly chose 200 strips from the total sample pool in all selected

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slices for examination at high magnification (Fig. 1). By doing preliminary work, we adjusted our sampling technique so that the experimental error was sensitive to a 10 percent change in cell number. For each slice examined, the area of the granular layer, areas of each selected sample, and areas of all complete and partial granule cell nuclear profiles (outlined in Fig. 1) within the samples were measured with a digitizer (Summagraphics) connected to a computer (Wang 2200) (accuracy within 0.75 percent).

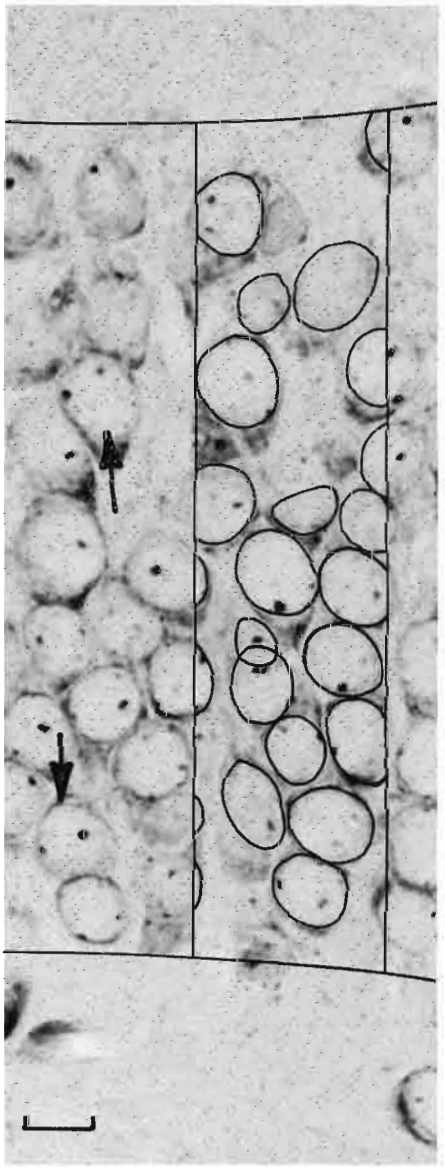

Fig. 1. A portion of the dorsal dentate granular layer in a 30-day-old rat showing a representative sample area. After this photograph was printed, the same region was relocated under high magnification, and all complete and partial granule cell nuclear profiles were outlined. The same procedure was followed for each of the 200 strips analyzed in this brain. Arrows indicate cell nuclei with double nucleoli (3-μm methacrylate slice stained with cresyl violet; scale bar is 10 μm).

These measurements provided the basis for the final estimation of V_t (*9*).

To estimate V_a in the ventral part of the granular layer, for example, the nuclear profiles completely contained in all ventral samples (between 700 to 1000 in each animal) were used to form empirical distributions of observed ventral granule cell nuclear sizes. Complete profiles contained in all dorsal samples (usually 2000 or more observations for each animal) were similarly processed. In both parts, V_a was estimated by applying the method of Hendry (*10*) to the empirical distributions.

Final cell numbers ranged from means of 890,146 at 30 days to 1,276,734 at 365 days (Fig. 2). The analysis of variance indicated the linear increase was due to age [$F(3, 12) = 34.16$, $P < .0001$]. Between 1 month and 1 year, there was a net gain of 43 percent (*11*), and cells were added at the approximate rate of 1,149 per day. Under the assumption that the left hippocampus had similar gains, approximately 770,000 granule cells originated between 30 and 365 days. During this time, the granular layer increased 35 percent in total volume (1.69 mm^3 at 30 days to 2.28-mm^3 at 365 days) in an age-related linear pattern [$F(3, 12) = 25.68$, $P < .0003$]. There was also an 18 percent decrease in V_a in the ventral part (from 664 μm^3 at 30 days to 562 μm^3 at 365 days) [$F(3, 12) = 14.39$, $P < .0026$], and ventral nuclear packing density increased by approximately 50,000 cells per cubic millimeter between 1 month and 1 year. In the dorsal part, V_a was considerably smaller (approximately 465 μm^3) at all ages and remained constant.

The absolute number of granule cells given here for 30-day-old Wistar rats is 42 percent greater than that reported by Schlessinger *et al.* (*5*) for 28-day-old Holtzman rats; genetic factors may be responsible, since Wimer *et al.* (*12*) found granule cell numbers to vary as much as 60 percent between strains of mice. Our range of values for adults aged 120 to 200 days are similar to those reported by Gaarskjaer (*6*) in Wistar rats weighing 200 to 300 g (0.99 million to 1.19 million). On the other hand, these results cannot be related to the estimate of 2.17 million granule cells recently reported in adult Wistar rats; the discrepancy is probably due to considerable variation in estimation procedures (*13*).

In the rat, granule cells in various brain regions increase during the postnatal period. However, neurogenesis stops by 21 days in the cerebellum (*14*), and the small increase observed in olfactory bulb granule cells between 30 and 365 days was not significant (*15*), even though olfactory bulb granule cells originate during the adult period (*3*). This report shows that neurogenesis of hippocampal granule cells in the adult brain is unique in that it substantially adds to the existing population. It is reasonable to assume that the addition of new neurons means that more postsynaptic sites are constantly being made available to those neurons supplying input to the dentate molecular layer.

Assigning a specific function to the hippocampus is still controversial. Many experiments show the hippocampus to be involved in short-term memory (*16*), especially spatial memory (*17*). Other studies implicate hippocampal activity in response inhibition (*18*), which develops in synchrony with the maturation of the dentate gyrus (*19*). The growing body of evidence for morphological and physiological plasticity of the dentate granule cells in both immature and mature animals (*20*) suggests that the dentate gyrus may be active during the brain's response to changes in the environment. Granule cells must play a pivotal role in total hippocampal function, since their elimination by early postnatal x-irradiation in rats gives behavioral deficits simi-

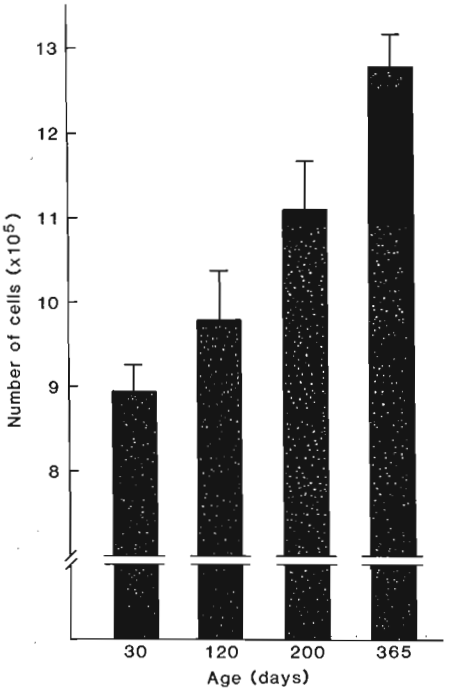

Fig. 2. The total number of granule cells in the dentate gyrus of the right hippocampus in rats aged 1 month to 1 year. Bars represent means of four animals plus standard error. Age and cell number are positively correlated [$r(14) = .85761$, $P < .0001$], and regression analysis shows significant linear numerical increases in the granule cell populations of older rats [$F(1, 14) = 38.93$, $P < .0001$].

lar to those seen after bilateral hippocampal lesions (*21*). The continued numerical increase of granule cells in the adult suggest that their influence on total hippocampal function grows with age.

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8. Pilot studies showed average nuclear volume to be consistently larger in the ventral than in the dorsal dentate granular layer. To improve accuracy of the final estimate, we separately determined the number of granule cell nuclei in dorsal and ventral parts. The part of the dentate gyrus lying posterolateral to the thalamus was designated "ventral," and the part remaining, lying either directly above or dorsolateral to the thalamus, "dorsal."
9. We considered the total area of granule cell nuclei in each ventral slice (A_t) to be approximately
$$A_t = (\Sigma A_n/\Sigma A_s)\,(A_g)$$
where A_n is the summed areas of all granule cell nuclear profiles, A_s is the summed sample areas, and A_g is the granular layer area. Finally, V_t was the summed products of A_t for each slice and the distance D to the next slice:
$$V_t = A_{t1}D_1 + A_{t2}D_2 + \ldots A_{ti}D_i$$
where i is the last slice to contain the ventral part of the granular layer; the same procedure was applied to all slices of the dorsal part. Since the material consisted of three-dimensional slices, not two-dimensional sections, V_t was inaccurately estimated as a result of the Holmes effect [E. R. Wiebel, *Int. Rev. Cytol.* **26**, 235 (1969); H. Elias, A. Hennig, D. E. Schwartz, *Phys. Rev.* **51**, 158 (1971)], and a correction factor was applied to the data.
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11. One of the 1-year-old animals had an abnormal total cell count 4.74 standard deviations below the mean of the other four animals in this age group. When this animal is included, the mean at 365 days is 1,206,209 ± 76,194, and the increase between 30 and 365 days was 35 percent [$F(3, 13) = 14.66$, $P < .0021$]. Since this animal was not representative of its age group, it was not included in the data of Fig. 2.
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22. We thank J. Altman for advice and encouragement; S. Evander, L. Ross, and K. Sandefur for the histology; C. Landon for the photography; M. Mucciarone for computer programming; and T. Chen, C. Hoover, J. Haux, and W. Stumbo for computer operation. This work was supported by grants BNS 79-21303 (to S.A.B.) and MCS-8102733 (to P.S.P.) from the National Science Foundation.

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