poglossal nucleus (*6*). There was variable subtotal Nissl dispersion beginning approximately 4 days after toxin application, with variable but incomplete cell loss, similar to that reported by others (*7*), beginning after 7 days. These changes after nerve transection were strikingly different in quality and timing from the changes observed after application of the toxic lectins.

To confirm that ricin was retrogradely transported, 0.5 μl of ricin-HRP conjugate (0.25 μg) was injected into the cervical vagus of three rats. After 24 to 48 hours, neuronal perikarya and processes were intensely labeled in the ipsilateral dorsal motor nucleus and nodose ganglion. Anterograde labeling of vagal sensory terminals in the nucleus of the tractus solitarius was not observed, suggesting that ricin killed nodose ganglion cells before the conjugate could be transported centrally. In five rats ricin-HRP conjugate (0.2 μg) was injected into the nucleus of the tractus solitarius. After 48 hours many cells were labeled in the nodose ganglia, but labeled neurons were rarely observed within the central nervous system. Although extensive necrosis occurred at the injection site and typical cytotoxic changes were seen in the nodose ganglia, no evidence of cytotoxicity was observed in central neurons known to project to the nucleus tractus solitarius.

Results obtained with ricin-HRP conjugate applied to the vagus are similar to observations made in postganglionic sympathetic neurons that have demonstrated retrograde axonal transport of ricin from the anterior chamber of the eye or submandibular salivary gland to the superior cervical ganglion (*1*, *8*). However, no permanent neuronal loss was reported (*1*, *8*).

Our study shows that ricin is retrogradely transported after direct application to a peripheral nerve. Neuronal death occurs after ricin reaches the perikaryon, and the resulting lesion is limited to neurons projecting to the injection site. The rapid (24 to 48 hours) and complete loss of Nissl substance after application of the toxic lectins is probably due to their action on polyribosomes (*2*, *9*).

The ability of sensory and motor axons to take up and retrogradely transport amounts of toxic lectin sufficient to kill the parent neuron should prove useful for permanently denervating selected target organs in the peripheral nervous system and for destroying the sensory and motor neurons whose axons project through a particular nerve. This process of suicide transport may also be adaptable to neurons with axons confined to the central nervous system. Local injection of a toxin into an appropriate terminal area of one set of collaterals may result in death of the parent cell body and of other collaterals. Neuronal death by suicide transport could be used to study adjustments made by axonal processes synapsing on the dying neurons. Moreover, by coupling the toxic moiety of ricin to a second molecule such as nerve growth factor, to another lectin, or to an antibody that is taken up only by certain neurons, it may be possible to selectively destroy specific classes of neurons.

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

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).

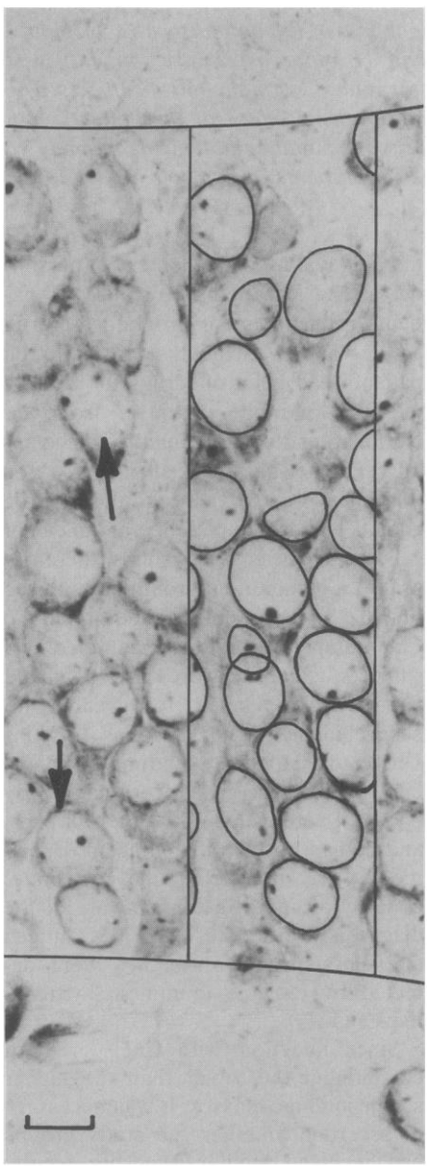

Fig. 1. A portion of the dorsal dentate granular layer in a 30-day-old rat showing a representative sample area. After this photograph was printed, the same region was relocated under high magnification, and all complete and partial granule cell nuclear profiles were outlined. The same procedure was 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-

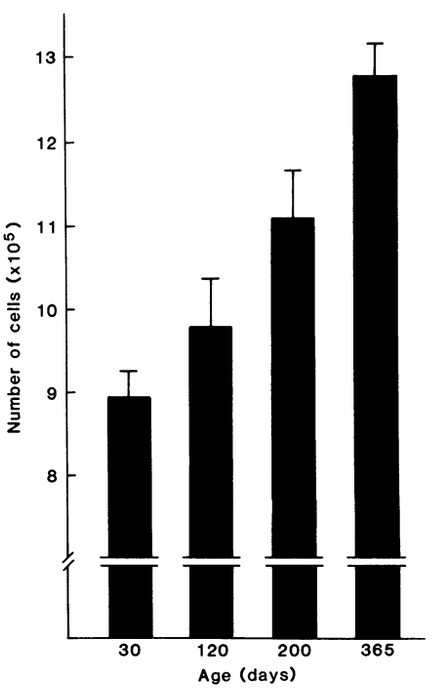

Fig. 2. The total number of granule cells in the dentate gyrus of the right hippocampus in rats aged 1 month to 1 year. Bars represent means of four animals plus standard error. Age and cell number are positively correlated [$r(14) = .85761$, $P < .0001$], and regression analysis shows significant linear 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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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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Cytomegalovirus Antibody in Cerebrospinal Fluid of Schizophrenic Patients Detected by Enzyme Immunoassay

Abstract. *By means of enzyme immunoassay techniques to detect the presence of antibody to cytomegalovirus, the cerebrospinal fluid of 178 patients with schizophrenia, 17 patients with bipolar disorders, and 11 other psychiatric patients was compared with that of 79 neurological patients and 41 normal control subjects. The cerebrospinal fluid of 20 of the schizophrenic patients and 3 of the patients with bipolar disorders showed significant increases in immunoglobulin M antibody to cytomegalovirus; no difference was found in patients on or off psychotropic medications.*

Viruses have come under increasing suspicion as possible causative agents in chronic central nervous system diseases such as multiple sclerosis, Parkinson's disease, and Alzheimer's disease. By means of an enhanced neutralization test, schizophrenic patients were found to have an increased ratio of cerebrospinal fluid (CSF) to serum antibody directed at cytomegalovirus (CMV) compared to normal controls (*1*). Cytomegalovirus has many characteristics that suggest it as a possible causative agent in schizophrenia; these characteristics include its known neurotropism, affinity for the limbic system, and potential for latency. Infection with CMV is also more prevalent in the lower than the higher social economic populations, as is schizophrenia (*2, 3*). Using enzyme immunoassay techniques, we have now studied CMV in a large group of psychiatric, neurological, and control individuals.

Samples of CSF were obtained from 178 patients with schizophrenia, 17 patients with bipolar disorders, 11 patients with other psychiatric disorders, 79 patients with neurological disorders, and 41 normal control subjects (*4*). Research Diagnostic Criteria were utilized for the diagnosis of schizophrenia and bipolar disorders (*5*). The other psychiatric patients were diagnosed as having dementia, personality disorders, and psychoneuroses. The neurological patients had a wide variety of diagnoses including demyelinating diseases, seizure disorders, back problems, and central nervous system infections. The control subjects consisted of 10 surgical patients undergoing spinal anesthesia at the National Naval Medical Center, 16 personnel at St. Elizabeths Hospital, 14 patients in an inpatient program for drug addicts at St. Elizabeths Hospital (the patients had been off drugs for 2 to 24 months), and one volunteer from the National Institutes of Health. The hospital personnel had worked on the wards for a mean of 4.6 years, and the ex–drug addicts had been inpatients for a mean of 1.1 years; these two groups therefore shared a common environment with the St. Elizabeths patients. In addition to the CSF samples from these adult control subjects, samples were also available from 38 children (ages 1 to 16 years) with suspected meningitis at Johns Hopkins University Hospital, Baltimore, Maryland; 14 of these cases were caused by bacterial pathogens, 3 by enteroviruses, and 21 had no identifiable agent. Specimens were intermixed and run under code by an investigator unaware of the diagnosis. Antibodies to CMV antigen and class-specific antibodies were detected by enzyme immunoassay techniques (*5–13*).

Since infection with CMV is more common in the lower than the higher socioeconomic classes, it was necessary to ascertain whether the study groups had received similar exposure to the virus. We therefore tested serum specimens from 60 of the schizophrenic patients and 26 of the normal adult controls for CMV antibody (*14*). No difference was found between the two groups, suggesting that exposure to this virus had been comparable.

Samples of CSF from the first 109 schizophrenic patients and 24 control

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