msec. The data were collected from one subject over a series of ten sessions. The data set at each intensity contained 160 EP's (16 conditions by ten replications), each based on averaging the EEG to 34 to 51 stimuli (depending on the intratrial list position).

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9. The storage component was orthogonal to a number of other uncorrelated EP components, including P300 and CNV-resolution, which were also found in the analyses. Thus, this storage component is not just a variant of well-known EP components, but rather, is orthogonal to them in a formal analysis. Also, this storage component was not correlated with eye movements or with the amount of alpha in the EEG.
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11. The order of number-relevant and letter-relevant runs was reversed for half the subjects. Practice trials on the primary task were given until ten consecutive trials were correct. The average performance on the primary task was 97.5 percent correct. The light intensity was approximately 2.8 log units above threshold for character recognition.
12. Percentage of correct responses were converted to probit scores (z-score units); 50 percent and 98 percent thus become 0.0 and +2.05 probit scores.
13. H. R. Bragdon collected the data in the EP experiment. D. Gershowitz and J. K. Martin collected the data in the behavioral memory-probe experiment. Supported in part by PHS grants EY01593 and EY01319 and contracts N00014-77-C-0037 and CNA SUB N00014-76-C-0001 from the Office of Naval Research.

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Exponential Decrease During Aging and Random Lifetime of Mouse Spermatogonial Stem Cells

Abstract. *Variation in the number of spermatogonial stem cells during the life-span of the mouse was examined by assaying the number of clonogenic cells, that is, spermatogenic stem cells, surviving known doses of radiation. The results indicated that the stem cell number decreased exponentially with age.*

In general, stem cells have been defined as undifferentiated cells with the capability of limited or unlimited self-renewal, although their real nature has not been well clarified (*1-4*). If spontaneous mutations are inevitable at every DNA replication or mitosis of mammalian cells in vitro or in vivo (*5*), and if stem cells have to be genetically stable, it seems unreasonable that they would have unrestricted infinite reproductivity. A finite lifetime of cells cultured from human embryos has been reported and has been considered to be a factor responsible for limited life-span and aging of organisms (*6*).

In the work described herein, we used a microcolony assay method for mouse spermatogonial stem cells (*7*) to determine the changes in stem cell number during the life-span of the mouse. The testes of C_3Hf/Bu male mice of various ages from our SPF (specific pathogen-free) breeding colony were irradiated from two opposing ^{137}Cs sources (1058 rad/min). Each mouse was confined in a Lucite box in air without anesthesia during irradiation. Details of the microcolony assay have been described elsewhere (*7*). Briefly, testes were removed and fixed in Bouin's solution 35 days after irradiation. Histological sections taken at the equator of each testis were stained with hematoxylin and eosin. Tubule cross sections showing regenerating seminiferous epithelium (that is, differentiated spermatogonia or cells at later stages of spermatogenesis) were scored with a light microscope (×100), while the total number of cross sections was counted with a projection microscope. On the assumption that one surviving stem cell can regenerate an island of seminiferous epithelium at any age, and since radiosensitivity did not vary significantly with age (Fig. 1), and the total number of tubules cross-sectioned remained constant, the surviving fraction after a certain dose reflects the initial number (prior to irradiation) of stem cells.

Figure 2 shows survival fractions of spermatogonial stem cells after irradiation of mice of various ages with either 1000 or 1200 rads. In both groups of mice the surviving fraction decreased exponentially with age between 7 and 122 weeks.

After Hayflick demonstrated (*6*) a lim-

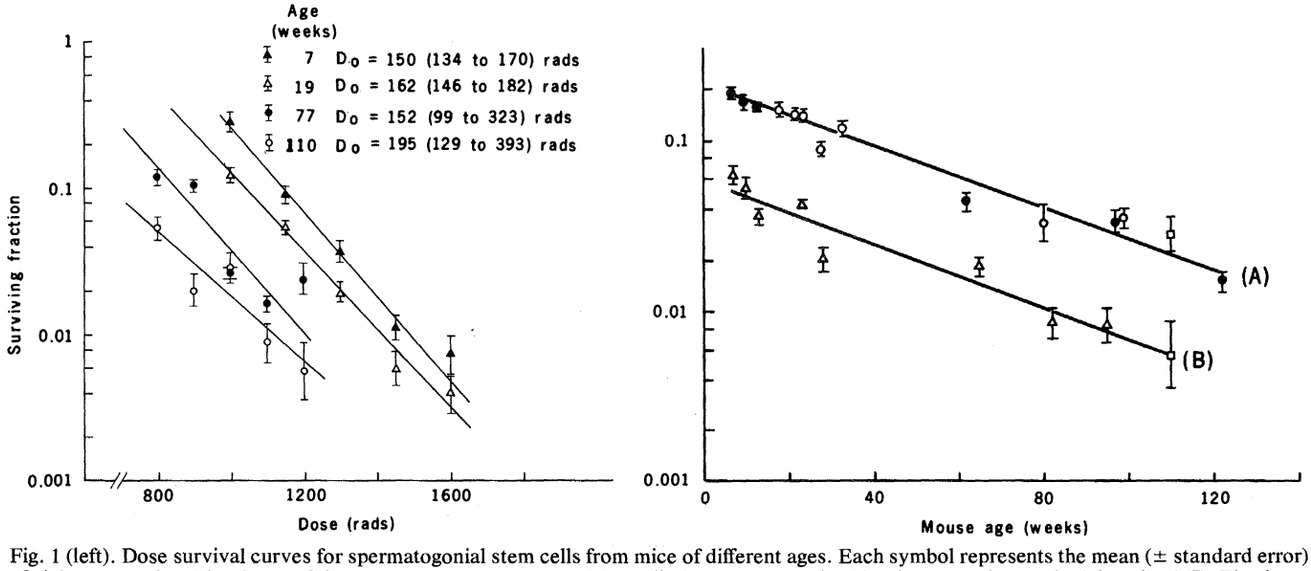

Fig. 1 (left). Dose survival curves for spermatogonial stem cells from mice of different ages. Each symbol represents the mean (± standard error) of eight to ten mice. The slopes of the curves and 95 percent confidence limits were determined by the regression method described (*7*). The doses (with 95 percent confidence limits) required to reduce survival by one natural logarithm (D_0) were 150 (134 to 170) rads at 7 weeks, 162 (146 to 182) rads at 19 weeks, 152 (99 to 323) rads at 77 weeks, and 195 (129 to 393) rads at 110 weeks. Fig. 2 (right). Fraction of tubular cross sections showing regeneration of seminiferous epithelium after irradiation with (A) 1000 rads or (B) 1200 rads at various ages. Different symbols indicate separate experiments. Each symbol represents the mean (± standard error) of ten to 15 mice. Lines were fitted by computer using a least-squares regression analysis. The times (with 95 percent confidence limits) required to reduce stem cell number by one natural logarithm were for line (A) 49 (44 to 56) weeks and for line (B) 47 (36 to 68) weeks.

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ited generation capacity for normal cells from human embryos in vitro, that is, about 50 generations, other workers made similar claims of a limited lifetime for hemopoietic (*1*), mammary (*2*), and skin epithelial (*3*) cells in vivo. It has been assumed that the number of stem cells in a tissue remains at a certain level for most of the life-span of an organism, and that a uniformly limited lifetime or renewability of stem cells determines the life-span of the body (*1-3, 6*). There are problems in these interpretations, mainly because of the artifacts, uncertainties, and possible selection mechanisms introduced by serial transplantation of tissues from host to host or by subculture. For the testis system described herein the cells are confined to one organ without transplantation.

Our results with mouse spermatogonial stem cells do not agree with conventional views about the stem cells and aging. They show that the number of spermatogonial stem cells in the mouse decreases exponentially with age, indicating that the lifetime of the mouse spermatogonial stem cells in situ is not uniformly specified but limited in a random manner.

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6-Hydroxydopamine and Anticholinergic Drugs

Schallert *et al.* (*1*) reported that depletion of brain catecholamines by the use of intraventricular injections of 6-hydroxydopamine (6-OHDA) resulted in an exaggerated response to anticholinergic drugs such as atropine and scopolamine. They interpreted this finding in terms of an interaction of acetylcholine with dopamine in the central nervous system. Since these authors used a dose and administration regime of 6-OHDA that depletes norepinephrine (NE) (*2*) as well as dopamine, we think it is premature to claim this as evidence for an interaction solely with dopamine. Schallert *et al.* (*1*) used a total dose of 500 μg of 6-OHDA, without first injecting desimipramine to prevent NE neuron destruction. Although they reported that the NE concentration at postmortem was depleted by only 11 percent, this assay was carried out on caudate-putamen, which is generally regarded as a blank region for NE. Certainly, on the basis of previous studies (*2*) in which this amount of 6-OHDA was used, a very severe NE depletion would be expected in regions such as hippocampus, cortex, and hypothalamus which do contain significant NE concentrations. Thus, the authors are not justified in attributing their effects to an acetylcholine-dopamine interaction as opposed to an acetylcholine-NE interaction.

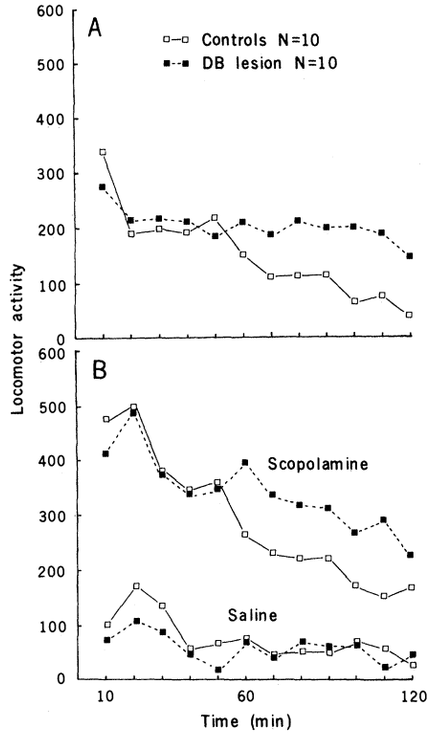

Fig. 1. (A) Locomotor activity of rats with 6-OHDA lesions of the dorsal bundle (*DB*). The activity was measured by means of photocells every 10 minutes for 2 hours. We used ten controls and ten noradrenaline-depleted rats injected intraperitoneally with atropine (10 mg/kg). (B) Locomotor activity in response to intraperitoneal injections of scopolamine (1 mg/kg) and saline.

This is important because we have obtained data that indicate that a pure NE depletion can enhance the locomotor stimulation in response to anticholinergics. We injected 4 μg of 6-OHDA dissolved in 2 μl of 0.9 percent saline into the fibers of the dorsal noradrenaline bundle in the mesencephalon (*3*). This resulted (Table 1) in a severe and permanent depletion of forebrain noradrenaline in cortex-hippocampus to less than 5 percent of controls, but there was no significant loss of striatal dopamine. Nonetheless, the locomotor response to atropine (Fig. 1A) or scopolamine (Fig. 1B) was significantly potentiated. We thus conclude that the effects reported by Schallert *et al.* (*1*) may not be due to the acetylcholine-dopamine interaction they suggest but to an interaction with NE instead. An acetylcholine-NE interaction is well established in the peripheral nervous system (*4*) and biochemical evidence has suggested that such also occurs in the central nervous system (*5*). Our data indicate that an acetylcholine-noradrenaline interaction must also be considered in the control of locomotor

Table 1. Regional amine assay values obtained at postmortem for control rats and rats injected with 6-OHDA. Values are means with standard error of the mean in nanograms of amine per gram wet weight of tissue.

Region	Controls	6-OHDA	Percentage*
Noradrenaline			
Cortex-hippocampus	264 ± 6	6 ± 1	2
Hypothalamus	2,240 ± 77	590 ± 87	26
Cerebellum	219 ± 12	271 ± 8	124
Spinal cord	255 ± 6	307 ± 12	120
Dopamine			
Striatum	13,170 ± 570	11,570 ± 1,190	88

*The percentage of control concentrations remaining in lesioned tissues.

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