Since the fusions are intraspecies, and in many cases involve highly aneuploid lines, it is not possible to perform detailed cytogenetic analyses. However, comparisons of total chromosome counts of hybrid clones with those of the parental cell lines indicate varying degrees of chromosome loss in the hybrids. The median chromosome number in the hybrids from the GM 639 × T98G and GM 639 × GM 639 fusions is very nearly the same as the sum of the median number for each of the parental cell lines. In contrast, the median chromosome number of hybrids from fusions of GM 639 with HeLa, HT 1080 21A, and HT 1080 is 15 to 30 less than the sum of the median chromosome numbers of the parents, but in all cases larger than the number for either parent. Thus, it appears that there is no correlation between the extent of chromosome loss and the proliferative potential of the hybrids. In addition, the hybrids are positive for T antigen and therefore retain chromosome 5 of the SV40-transformed parent to which the viral genome has been mapped (*8*).

We conclude that the phenotype of immortality is recessive in hybrids with normal human cells and that complementation between immortal parent cells can result in hybrids with limited division potential. The data indicate that changes in two or more different events (or sets of events) occurring in the genetic program that limits the division of normal cells can result in immortality. The changes in the normal cell genome that lead to SV40 virus–mediated immortality are different from those occurring in the tumor-derived cell lines studied, with the possible exception of HT 1080 and its subclone; changes leading to immortality in HT 1080 may be the same as those mediated by SV40 virus. The fact that hybrids formed from fusion of 143B TK^- cells with SV40-transformed or HT 1080 cells showed limited proliferation is compatible with this possibility.

Our results support the hypothesis that limited proliferation is a result of a rigorously programmed series of events and that immortality is caused by certain changes—recessive in hybrids—in these events. The results argue strongly against the hypotheses that errors in the protein-synthesizing machinery of cells or recessive mutations are responsible for limited division in normal cells (*9*). The hypotheses suggest that the probability of these events occurring is decreased or eliminated in immortal cells. If this were true, fusion of immortal cell lines should yield only immortal hybrids, which we have experimentally found not to be the case. Smith and Lumpkin (*10*) proposed a hypothesis and model involving changes in gene expression to explain the mechanisms that limit the division potential of normal human cells in vitro. On the basis of this model, one would expect that cells could become immortal by at least two different mechanisms. The results we have described are compatible with this model.

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7. The hybrid cultures were grown in 25-cm^2 tissue culture flasks. At each subculture the inoculum was 1.25×10^5 cells. Thus, when the hybrids entered the period of slow growth or division cessation, each flask contained about 1×10^5 cells. When foci of dividing cells appeared, there were generally one or two per flask. We therefore estimated the frequency of this occurrence as one in 10^5 cells. The cultures in which such foci appeared were subcultured through an additional 100 population divisions, at which time we assumed the culture would divide indefinitely.
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Aged Rats: Recovery of Motor Impairments by Intrastriatal Nigral Grafts

Abstract. *Dissociated cell suspensions, prepared from the substantia nigra and septal regions of rat embryos, can be grafted to the depths of the caudate-putamen and hippocampus of aged rats. The grafts were rich in dopamine-containing and acetylcholinesterase-positive neurons and had produced extensive new dopaminergic and cholinergic terminal networks in the host neostriatum and hippocampus, respectively. The intrastriatal dopaminergic grafts were associated with a significant improvement in motor coordination in the aged rats. This result suggests that the intracerebral grafting technique may provide a new tool for exploring the role of dopaminergic and cholinergic deficits in the neurological and behavioral impairments associated with aging.*

Aged rats, like aged humans, show a decline in brain functions, such as sensorimotor coordination (*1–3*) and learning and memory (*4, 5*). Recent experiments have attempted to correlate these deficits with age-dependent impairments in synaptic transmission of specific neurotransmitter systems. Thus, for example, deficits of sensorimotor coordination (*2*) and swimming abilities (*3*) in aging rats are similar to those seen after dopamine-depleting brain lesions (*6*), and they may be reduced by the administration of dopamine receptor agonists (*3*). Similarly, the age-related decline in memory and learning (*4, 5*) are reminiscent of the deficits seen after lesions to the septo-hippocampal system (*7*) or after administration of anticholinergic drugs (*8*); some positive results have been obtained in potentiating memory performance in aged rats and humans after administering cholinergic precursors and cholinesterase inhibitors (*4, 9*).

These recent data suggest that impairments in dopaminergic and cholinergic neurotransmission may contribute importantly to the decline in sensorimotor and cognitive function, respectively, associated with the aging process. We have previously shown that lesion-induced impairments of dopaminergic and cholinergic transmission in young rats can be partially reversed both biochemically (*10, 11*) and behaviorally (*12, 13*) by grafts of embryonic dopaminergic or cholinergic neurons; recent reports suggest that neuronal transplants can survive also in the aged rat brain (*14*). We now report that mesencephalic dopaminergic neurons and septal cholinergic neurons, taken from rat embryos, can be grafted with excellent survival rates to the neostriatum and hippocampus, respectively, in the brains of aged rats, and that the intrastriatal dopamine-rich transplants can ameliorate the impairments in motor coordination seen in these animals.

We studied female Sprague-Dawley rats (Anticimex, Stockholm) housed in group cages of three to eight animals

under a normal light-dark cycle, and with free access to food and water. Retired breeders ($N = 40$) were purchased when 9 to 11 months of age and kept in a clean, controlled environment for a further 12 months before being tested. A total of 31 rats survived to 21 to 23 months of age: 11 received bilateral grafts of embryonic septum into the dorsal hippocampus (*15, 16*), 8 received bilateral grafts of embryonic substantia nigra into the caudate-putamen (*15, 17*), and the remaining 12 constituted the aged control group. Twelve young adult rats were purchased at 8 weeks of age and allowed 2 weeks to adapt in the new environment before beginning testing in parallel with the aged groups.

Behavioral testing was conducted on all rats immediately before and 11 to 14 weeks after transplantation surgery. By the second phase of testing, 8 rats with septal grafts, 9 aged controls, all 8 rats with nigral grafts, and 12 young controls remained alive.

Motor coordination skills were assessed on four measures adapted from the battery described by Wallace *et al.* (*2*): (i) the ability to maintain balance on, and successfully reach, a safety platform at either end of a narrow bridge of square cross section; (ii) the ability to maintain balance on a similar bridge of round cross section; (iii) the duration for which the animal could sustain its own weight clinging suspended from a taut wire; and (iv) the ability to descend in a coordinated manner on a vertical pole covered with wire mesh. A maximum latency of 120 seconds was allowed on each test before the rat was removed. Before transplantation, the aged rats were significantly impaired with respect to the young controls on all four measures. On the square and round bridges, young rats have no difficulty walking and exploring along the rod, and they generally reach the platform within 30 to 60 seconds. The aged rats, by contrast, had greater difficulties maintaining balance. Most animals rapidly fell off, or alternatively lay on the rod without attempting to walk and clung on tightly either with all four paws or with the forepaws while the hindpaws hung freely (*2*). Twelve weeks after transplantation, the aged rats with nigral grafts, but not the aged control or septal grafted rats, had significantly improved their balance and limb coordination on both bridges (Fig. 1, A and B). Typically, they could walk along the bridge without falling, manifested gait and posture similar to the young rats, and fell less frequently.

The aged rats showed no difference between groups in either body weight or in latency to fall from the taut wire (Fig. 1C). This suggests that the differences on the two bridges were not attributable to nonspecific differences in weight or strength of the animals. On the pole covered with wire mesh the aged rats descended more rapidly than the young controls, frequently falling, slipping, or sliding down backward. Although the aged rats with nigral grafts showed a tendency to be improved on this task, they did not differ significantly from the other aged groups.

Locomotor activity was measured before transplantation and 11 weeks after in an automoted open field in which the floor was traversed by two perpendicular rows of photocell beams (*18*). As reported by others (*1, 19*), the aged rats displayed reduced whole-body locomotion with respect to young controls, but the transplants had no significant effect on this hypoactivity (Fig. 1D).

The swimming abilities of the rats were assessed 12 weeks after transplantation according to the 0–3 rating scales for efficiency and vigor described by Marshall and Berrios (*3*). We found no significant differences between the young and aged rats, with the exception of three rats with septal grafts that adopted a frantic corkscrew-like swimming below the surface and that needed to be rescued as soon as placed in the water. Thus, in tests both before and after transplantation, all animals initially swam efficiently (3 on a 3-point scale) but less so after 15 minutes (1.5); on the measure of swimming vigor the initial values of close to 3 declined to a mean of

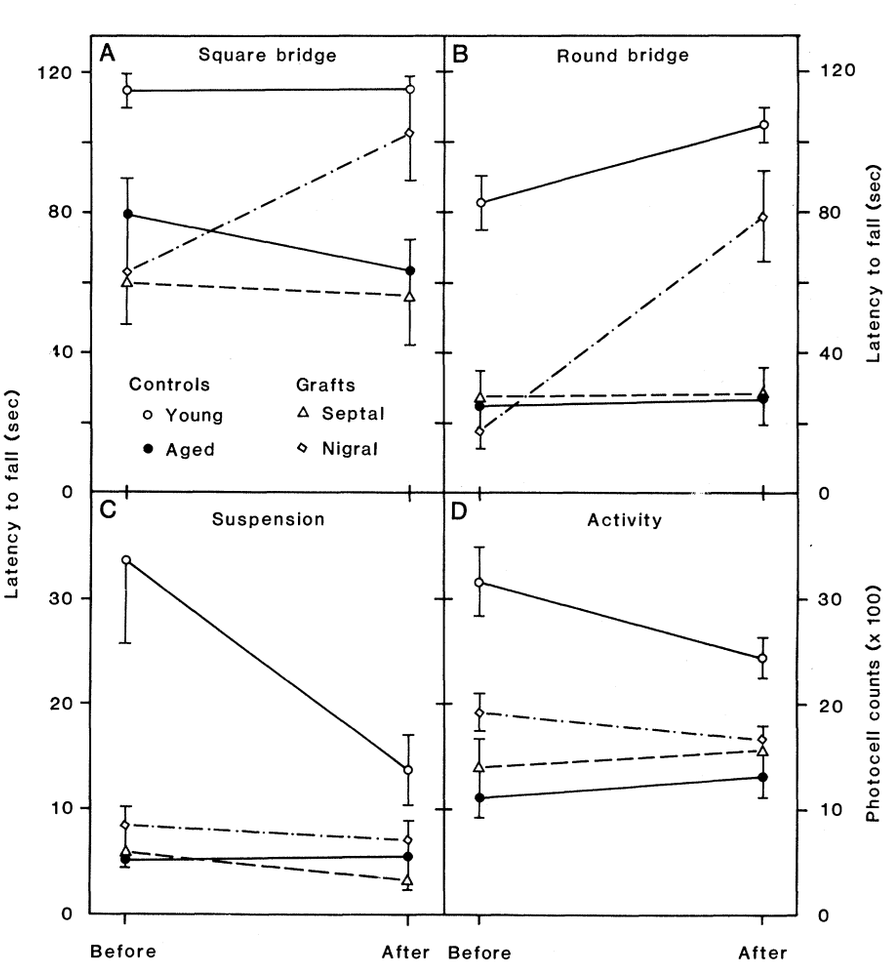

Fig. 1. Motor performance. Each point represents the group mean values on each test, and vertical bars indicate standard errors of the mean. Analyses of variance used 3 and 33 degrees of freedom. (A) Latency to fall from the square bridge. After transplantation, aged rats with nigral grafts improved their performance significantly [main group effect, $F = 9.26$, $P < 0.001$; group-by-test interaction, $F = 3.93$, $P < 0.05$]. (B) Latency to fall from the round bridge [main group effect, $F = 23.52$, $P < 0.001$; group-by-test interaction, $F = 8.35$, $P < 0.001$]. (C) Suspension from a taut wire. Neither graft influenced performance of the aged recipients [main group effect, $F = 6.11$, $P < 0.01$; group-by-test interaction, $F = 6.73$, $P < 0.01$]. (D) Locomotor activity counts in automated open field. Aged rats were less active than young controls, but no significant differences were seen between aged rats with or without transplants [main group effect, $F = 16.67$, $P < 0.001$; group-by-test interaction, $F = 3.07$, $P < 0.05$].

approximately 1 by the end of the test.

The brains from the animals given nigral transplants and half of the controls were processed for catecholamine fluorescence histochemistry (*20*), and the brains from the septal animals and remaining controls were stained for acetylcholinesterase (AChE) (*21*). In seven nigral rats the intrinsic nigrostriatal pathway was removed unilaterally by 6-hydroxydopamine (6-OHDA) [8 μg in 4 μl (*10, 12*)] and in eight septal rats the septohippocampal cholinergic pathway was sectioned by a unilateral fimbria-fornix lesion (*11, 13*) 6 to 10 days before they were killed, in order to remove the intrinsic dopamine and AChE-positive innervations of the grafted caudate-putamen and hippocampus, respectively, and thus allow observations of fiber outgrowth from the grafts.

Surviving grafts were found on both sides in all animals with grafts (Fig. 2). Graft survival rate, volume, and histological appearance were not appreciably different from what have previously been observed after grafting in young adult hosts (*13, 15*). The nigral grafts contained many catecholamine-fluorescent (presumably dopamine-containing) neurons (Fig. 2, A and B); in those animals in which the intrinsic striatal dopaminergic innervation had been completely removed before death, the grafted neurons had produced a rich fluorescent dopamine-containing fiber network in the host caudate-putamen that had grown in a halo extending in a gradient about 1 to 2 mm out from the graft (Fig. 2A). The completeness of the 6-OHDA lesion of the intrinsic dopamine pathway was assessed by observing (i) that areas in the striatum distal to the transplant were without catecholamine histofluorescence and (ii) that three rats without transplants but with identical 6-OHDA lesions were without catecholamine fluorescence throughout the entire striatum. Similarly, the septal grafts contained many AChE-positive neurons (Fig. 2E) and had produced an extensive AChE-positive fiber network in the host hippocampus, as revealed in the rats with fimbria-fornix lesions (Fig. 2, D and F).

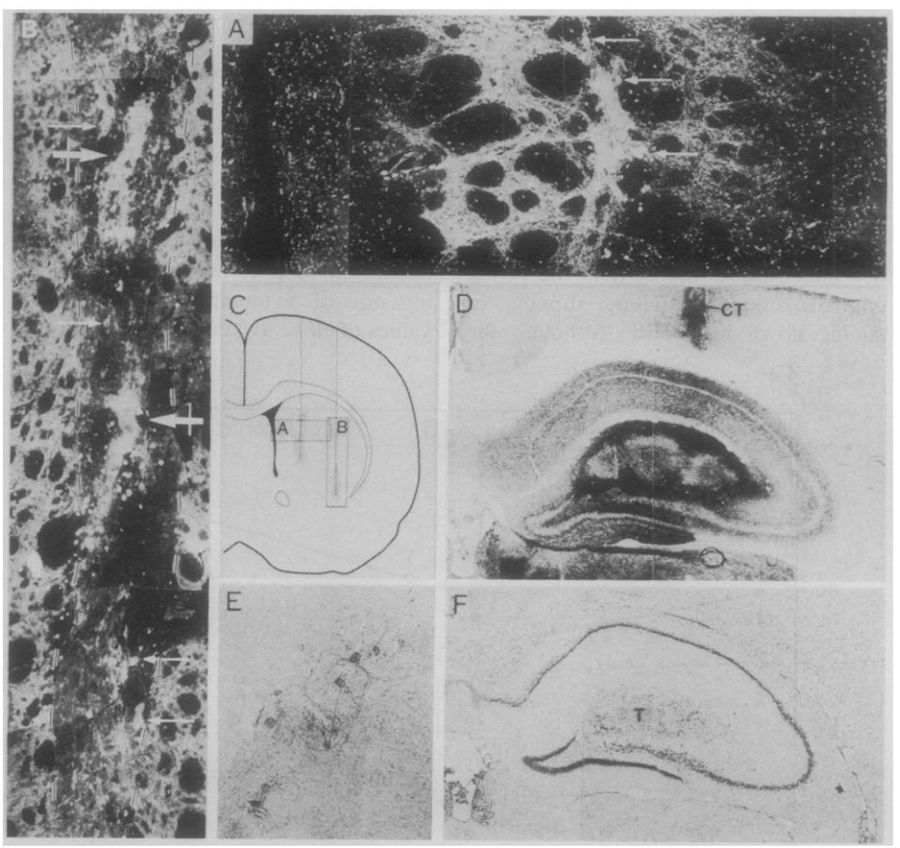

Fig. 2. Photomontages of surviving grafts of nigral cell suspensions, injected into the caudate-putamen (A and B) and septal suspensions into the hippocampus (D–F) of aged rats. (A) The intrinsic dopaminergic innervation was completely removed by an ipsilateral 6-OHDA lesion 10 days before death to enable graft-derived fiber outgrowth to be visualized. Surviving dopamine-containing cells of the graft can be seen as a strongly fluorescent cell cluster in the center, with a few individual cells visible in its periphery (arrows). The outgrowing dopamine fibers have formed a new fluorescent terminal network extending approximately 1 to 2 mm into the host caudate-putamen around the graft. (B) Nigral graft from another animal, showing surviving dopamine-containing neurons and fibers in the graft (outlined by dashes) and fibers extending into the intact fluorescent terminal network in the surrounding nondenervated caudate-putamen of the host. The grafted dopamine-containing neurons are seen both in densely packed aggregates (large arrows) and as individual cells (small arrows). The location and size of these montages is indicated in (C). (D–F) Septal transplant (*T*) in the dorsal hippocampal formation in an AChE-stained section (D) and in an adjacent section stained with cresyl violet (F). The host hippocampus was denervated of its normal AChE-positive innervation by a complete aspirative lesion of the ipsilateral fornix-fimbria 6 days before death. Thus, the AChE-positive staining in the graft and in the host hippocampus (D) represents fibers originating in the graft. *CT* indicates the track of the injection needle through the overlying cortex. (E) Surviving AChE-positive neurons in an intrahippocampal graft of septal cell suspension in an aged rat treated with diisopropyl-fluorophosphate 18 hours before death (*21*). This treatment abolishes all fiber staining and makes the AChE-positive cell bodies and proximal processes more deeply stained.

These results demonstrate that dissociated cell suspensions of embryonic nigral or septal neurons can be implanted into the depth of the brain in aged recipient rats, can survive for a long period, and that such grafted neurons can reinnervate their normal target areas in the absence of any denervating lesions. Moreover, the improvement in motor coordination abilities seen in the nigral, but not the septal, rats provides the first evidence that intracerebral neural implants may be able to restore age-related impairments in neurotransmission in specific neuronal systems. Nigral and septal suspension grafts thus provide new possibilities to explore the role of dopaminergic and cholinergic deficits in the neurological and behavioral impairments associated with advanced age; they may also offer a new experimental approach to functional restitution in animal models of aging and dementia.

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16. Septal cell suspensions were prepared by dissection of developing septal tissue from the ventral forebrain of embryonic donor rats (between the embryonic ages of days E14 and E16) of the same inbred strain. The tissue from 10 to 12 fetuses was collected in 0.6 percent glucose-saline in room temperature, incubated in 0.6 percent glucose and 0.1 percent trypsin-saline for 20 minutes at 37°C, and washed and mechanically dissociated in 0.1 ml of glucose-saline to form a milky cell suspension. Aged rats were anesthetized with 1.25 ml (per kilogram of body weight) of Rompun:Ketalar in a 1:5 ratio, and received three stereotaxic injections of 3-μl portions of the cell suspensions into the hippocampus on each side. Injection coordinates were: A (anterior) = 4.5 mm from interaural line, L (lateral) = ±3.5 mm, V (ventral) = 3.0 mm below dura; A = 3.0 mm, L = ±3.7 mm, V = 3.7 mm; and A = +3.0 mm, L = 4.8 mm, V = 5.7 mm. The incisor bar was set at 0 mm.
17. Nigral cell suspensions were prepared from E13 and E14 embryos, with tissue dissected from the ventral mesencephalic region containing the developinig substantia nigra–ventral tegmental area. Cell suspensions and surgery on the aged rats were conducted identically to the septal suspension injections. Each rat received six 2-μl injections bilaterally, stereotaxically aimed at the head of the caudate-putamen. Injection coordinates were: A = 1.0 mm from bregma, L = ±2.5 mm, and V = 3.5 and 5.2 mm below dura; A = +1.0 mm, L = ±3.5 mm, V = 5.0 and 6.5 mm; and A = −0.2 mm, L = ±3.8 mm, V = 4.0 and 6.0 mm, with the incisor bar set at 0 mm. The locations of the two rostral injections are illustrated in Fig. 2C.
18. Activity tests were conducted in a bank of six automated hole boxes. Each box was constructed of gray plastic 100 by 100 cm^2 with walls 60-cm high. The box was traversed by eight photocell beams, each 2 cm above the floor, at 20 cm intervals and parallel to each wall. Additionally, four rows of four 4-cm circular holes were cut into the floor at 20-cm intervals. Four additional photocell beams passed under the floor 1 cm below each hole to record nose pokes. Each rat received a single 60-minute test. The rat was placed in the box, and locomotor activity (interruptions of the upper photocell beams) and exploratory nose pokes (interruptions of the lower photocell beams) were automatically recorded by on-line connection to an ABC80 microprocessor. Although photocell counts were recorded in 3-minute time bins, only the 60-minute total has been included in the analysis; nose pokes were not analyzed further since young and old rats did not differ on this measure.
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Haloperidol-Induced Plasticity of Axon Terminals in Rat Substantia Nigra

Abstract. *An electron micrographic morphometric analysis of nerve endings in substantia nigra of rats repeatedly treated with haloperidol was performed. Although most parameters showed no difference, drug-treated animals exhibited a significant shift in the distribution of relative numbers of axon terminals, suggesting neuroleptic-induced axon-collateral sprouting.*

Various experimental studies have lead to the conclusion that dopamine receptor blockade is the mechanism of action of antipsychotic agents (*1*). Neuroleptic effects on the "dopamine system" include not only antipsychotic actions but also extrapyramidal movement syndromes (*2*). Permanent alterations of striatal and cortical integration following cessation of neuroleptic treatment have been inferred from clinical observations of tardive dyskinesia (*3*) and the so-called supersensitivity psychosis (*4*), respectively. These lingering effects of antipsychotic medication in some patients raise the possibility that these agents may induce structural changes in the synaptic organization of brain regions involved in dopamine-controlled mechanisms. A few studies to date investigating morphological changes associated with neuroleptic medication have used light microscopic approaches to suggest a decrease in the number of dopaminergic cells in substantia nigra (SN) (*5*). Since changes in central integration reflecting structural reorganization at the synaptic level could only be revealed by a quantitative electron microscopic (EM) analysis, the study here reported was done by the use of quantitative morphometric assessments at the EM level (*6*) to examine the synaptic relationships in SN of rats with chronic neuroleptic treatment.

Male Sprague-Dawley rats (200 ± 5 g) were used in this study. Experimental drug-treated rats were injected daily for 16 weeks with haloperidol (3 mg/kg, based on the daily body weight; McNeil Laboratories) dissolved in 0.02 m*M* lactic acid. Control animals were injected with appropriate volumes of lactic acid alone. A detailed description of handling, treatment, and behavioral changes has been presented elsewhere (*7*). At the end of the 16-week interval, all animals were anesthetized with Chloropent (1.0 ml per 300 g of body weight) and perfused intracardially (*8*). The SN, excised using a Zeiss OpMi-1 surgical microscope, was postfixed with 1.5 percent osmium tetroxide in 0.1*M* cacodylate buffer (*p*H 7.4), stained en bloc with 2 percent uranyl acetate, dehydrated with a graded series of ethanol and propylene oxide, and embedded in Spurr's epoxy resin. Thin (1-μm) sections were stained with toluidine blue to confirm the orientation of the SN. In addition, the numerical density and volume of dopaminergic and nondopaminergic neurons in the pars compacta distinguished by cytological criteria (*8*), were determined using a camera lucida projection system attached to a Zeiss light microscope. For neuron size, ten cells were obtained from each animal for a total of 40 control and 40 haloperidol-treated cell samples.

Ultrathin sections (30 to 40 nm thick, silver-gray) were counterstained with lead citrate, and viewed with a Siemens Elmiskop electron microscope. The EM morphometric analysis of SN was performed "blindly" on cross-sectional profiles of dendrites in the transitional zone between the pars compacta and the pars reticulata, which were in synaptic relationship with at least one axon terminal (Fig. 1A). A minimum of 12 dendrite cross-sectional profiles were obtained for each control ($N = 4$) and haloperidol-treated ($N = 4$) rat. The data shown in Fig. 1 represent 51 control and 48 drug-treated dendrite samples with their associated terminals ($N = 166$ and 187, respectively). The presence of a synapse was considered necessary to permit the definitive differentiation of a dendrite from glial processes. Number and area of dendrites and axon terminals, as well as the numerical density of synaptic vesicles, were determined for each sample according to a modification (*9*) of standard stereological techniques (*6*). These data were expressed as cross-sectional area of dendrites (caliber), number of axon terminals per dendrite, axon terminal volume, and number of synaptic vesicles per unit volume of axon terminal (synaptic vesicle density). A mean and standard error of the mean was obtained for each variable for the respective animals in each group, and these were used to assess the consistency of findings from animal to animal. In all cases, the values obtained for the animals in each experimental group showed good agreement.

A distribution curve for each parameter was initially plotted for experimental and control groups to determine whether