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The Effects of Haloperidol on Synaptic Patterns in the Rat Striatum

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A morphometric analysis of the corpus striatum of rats chronically treated with haloperidol was performed at the light and electron microscopic levels. Although the density of striatal neurons was unchanged in the haloperidol-treated group, there was a small increase in neuronal size (13%). This change in cell size was paralleled by a trend towards larger dendrite calibres occurring in the drug-treated animals. The distribution curve for axon terminal size indicated that 12% of the overall population was shifted from a range with a median size of $0.8 \mu\text{m}^2$ to one with $1.6 \mu\text{m}^2$ in the drug-treated group. This increase in size of some striatal terminals was accompanied by a concomitant increase in numbers of their associated synaptic vesicles, resulting in a similar density of vesicles for both control and drug-treated animals.

INTRODUCTION

Various clinical observations regarding the actions of neuroleptic drugs have led to conjectures that these agents may induce structural alterations in the brain. Some of these observations include: (1) the time-course for full development of antipsychotic effect of the neuroleptics may be as long as several weeks; and (2) the occurrence of tardive dyskinesia, an extra-pyramidal movement disorder associated with neuroleptic use, can persist indefinitely after the drug has been stopped. Recent animal studies indicate that increased dopamine receptor binding itself is not responsible for neuroleptic-induced oral dyskinesia, since equal increases were found with or without tardive dyskinesia. This finding suggests that 'other factors' must mediate this effect²¹. Support for this 'factor' being a structural one has recently come from our morphometric analysis of synaptic connections in the substantia nigra of rats⁵. Despite the absence of detectable neuroleptic-related changes at the light microscopic level, electron microscopic (EM) analysis of synaptic connections in these animals revealed a shift toward increased numbers of

axo-dendritic terminals occurring in the haloperidol-treated group. This latter change was traced to a single morphological sub-type of axon terminal for which neither the transmitter nor the site of origin has as yet been determined. The data, nonetheless, raised the question of whether synaptic changes similar to those observed in the substantia nigra might be found in other brain regions involved with the dopamine system. Theoretically, the neuroleptic drugs might induce non-specific changes in synapses regardless of their location. Alternatively, these agents might produce different alterations which reflect region-specific patterns of integration for the dopamine system.

The extension of analyses which were performed in the substantia nigra to the rat striatum represents a logical approach to resolving this question. This report describes a detailed EM morphometric analysis of axodendritic synapses in the striatum of control and haloperidol-treated rats. This analysis was also performed at the light microscopic level to determine neuronal size and density, since some investigators have reported neuronal cell loss in striatum of rats treated for 16 weeks with neuroleptic agents¹⁸.

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METHODS

Animals

Male Sprague-Dawley rats weighing 200 ± 5 g were used for the studies. The rats ($n = 4$) were injected daily with haloperidol (3 mg/kg, based on the daily body weight) which was dissolved in 0.02 mM lactic acid for a period of 16 weeks. Control animals ($n = 4$) were injected with appropriate volumes of lactic acid alone. A detailed description of animal handling has been presented elsewhere⁹.

Tissue handling

At the end of the 16 week interval, all animals were anesthetized with Chlorpent (1.0 ml/300 g body weight) and perfused intracardially according to the two-step technique of Peters¹⁹. Just prior to perfusion, 0.1 ml of 2% sodium nitrite and heparin, 1000 USP units/ml, (1:1) were injected directly into the left ventricle. A fixative containing 1% formaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, was then perfused through the heart. Following 10 min of perfusion with this dilute fixative, a concentrated mixture containing 4% formaldehyde and 5% glutaraldehyde was then perfused for 10 min. The brains were left overnight in situ at 4 °C. The following day, the brains were removed from the calvarium and portions of the caudate nucleus were excised using a Zeiss OpMi-1 surgical microscope. The caudate tissues were post-fixed with 1.5% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.4, stained en bloc with 2% uranyl acetate, dehydrated with a graded series of ethanol and propylene oxide and embedded in Spurr epoxy resin. The tissue was sectioned using a DuPont-Sorvall MT-2 ultramicrotome. Thin sections (1 μ m) were stained with toluidine blue. Ultra-thin sections (silver) were counterstained with lead citrate and viewed with a Siemens Elmiskop electron microscope.

Morphometric analysis

Light microscopy. Toluidine blue-stained thin sections were viewed with a Zeiss light microscope. Random fields of neuron somata within the caudate nucleus were localized among the bundles of myelinated fibers which characteristically occur within this brain region (Fig. 1). A reticule grid was aligned within the fields so that the myelinated axon bundles

were excluded from the sample. The number of neuron somata within each field were counted and expressed as the number of cells per square micron of tissue. Three such samples were obtained for each animal. Criteria used to distinguish neurons from glia were the presence of nucleoli and dendrites. Astroglial cells were readily identified due to their very dark appearance with toluidine blue staining. Oligodendroglia, on the other hand, are difficult to distinguish from neuron somata and likely introduce some error into the counting procedure. For the purpose of comparison between control and haloperidol-treated groups, however, this consideration is not a significant one since the counting was performed by one investigator. The rater was known, on the basis of preliminary studies, to have a high degree of consistency in distinguishing oligodendroglia from neurons in repetitive samples. The counting error incurred from oligodendroglia, therefore, was assumed to be a consistent one for both experimental groups. The neuronal density determined in this manner showed a normal distribution (data not shown), and the two-tailed Student's *t*-test was, therefore, used to test the significance of differences between control and drug-treated groups.

The area of individual neuron somata in the caudate was determined using tracings of camera lucida projections taken at a magnification of 500 \times . The reticule field was aligned at a low power (45 \times) as described for the density determination above. The magnification was then raised to 500 \times and the first 10 neurons encountered were traced. These tracings were then entered into a Perkin Elmer 3220 Computer using a Summagraphics digitizing board. The area of these tracings was integrated and a mean and standard deviation for each sample obtained. The means and standard deviations for each of the 4 animals within the two experimental groups were then combined and a two-tailed Student's *t*-test performed.

Electron microscopy. Cross-sectional profiles of dendrites were selected for sampling since they provide a consistent unit for measurement of dendrite calibre, as well as determination of the number of axon terminals per dendrite (Fig. 2). Other parameters assessed in these cross-sectional dendritic profiles were the volume of each axon terminal, its respective number of synaptic vesicles expressed as a

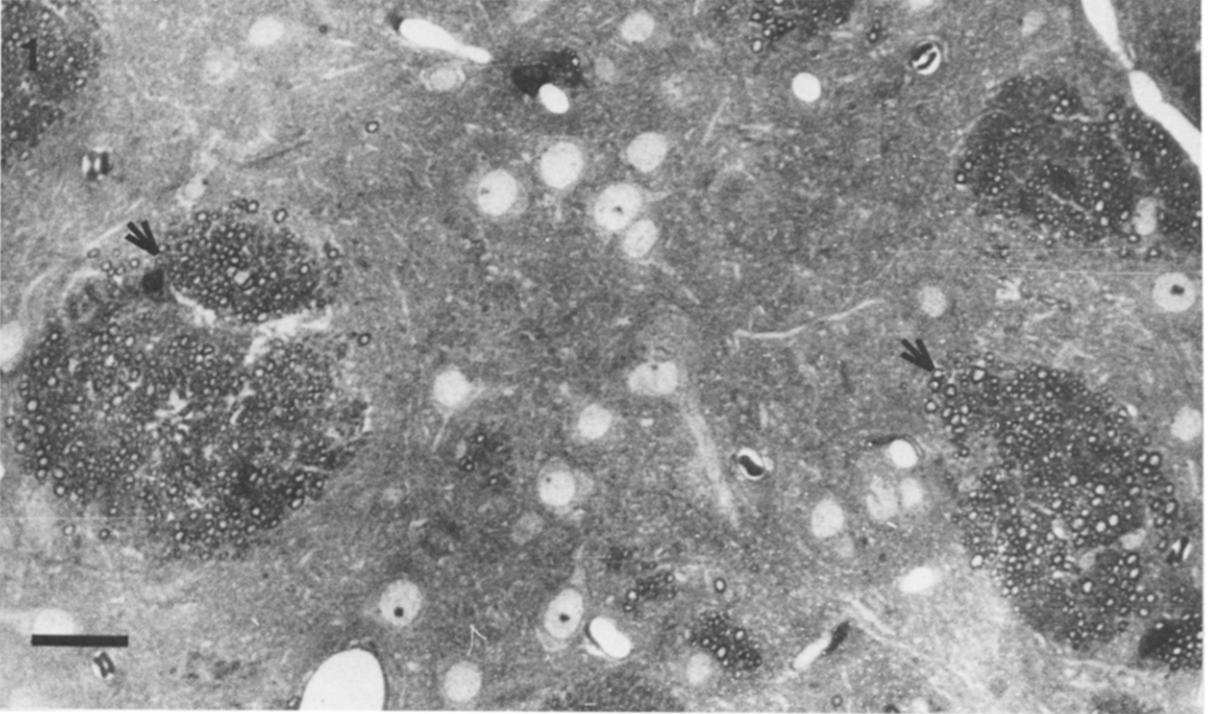


Fig. 1. A low-power bright-field light photomicrograph of rat striatum which was thin sectioned ($1\ \mu\text{m}$) and stained with toluidine blue. There are characteristic large bundles (arrows) of myelinated fibers seen in cross-section. Dispersed among these bundles are regions containing neuronal cell bodies and neuropil. Bar = $10\ \mu\text{m}$.

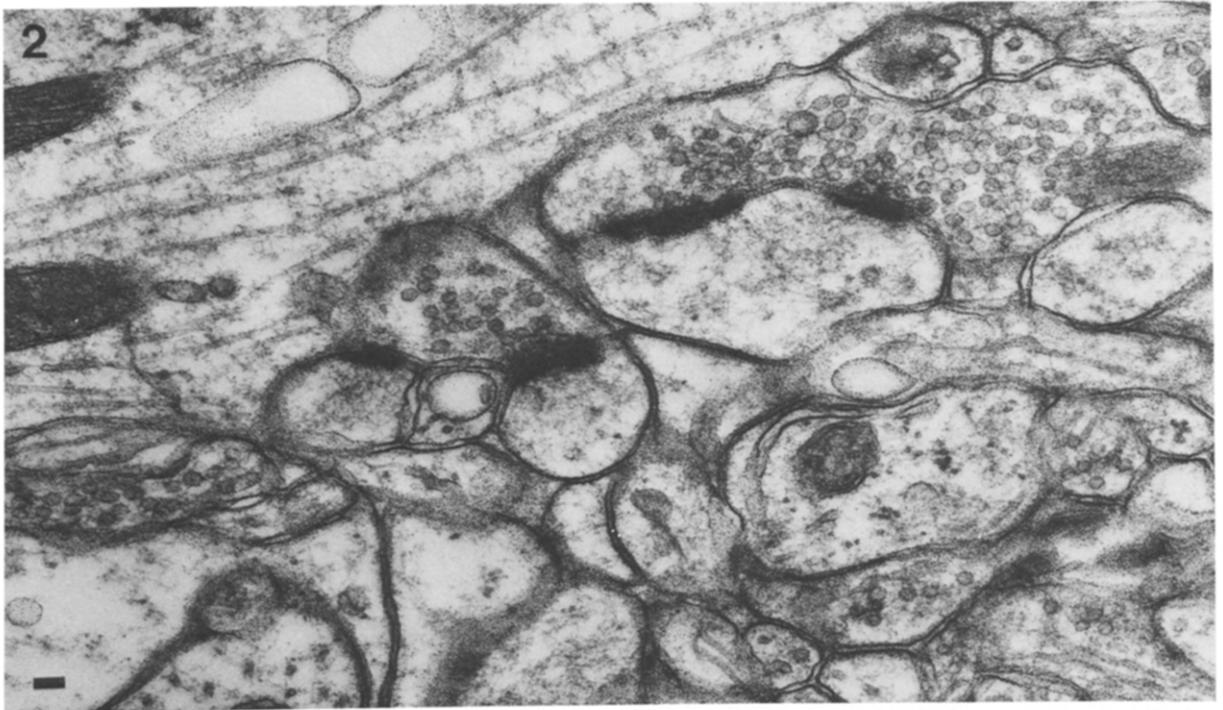


Fig. 2. Electron micrograph of a dendritic cross-sectional profile and an associated axon terminal. Bar = $0.1\ \mu\text{m}$.

density (number per unit volume of terminal), and the length of the contact between terminal and dendrite. The synaptic samples were obtained from fields similar to those described above for light microscopic determination of neuronal density and size. The sampling of dendrites in such a field was begun in close proximity to a bundle of myelinated axons and proceeded in a linear trajectory toward the second bundle. For the first round of sampling, 12 successive fields of neuropil containing a dendritic profile were photographed at a magnification of $9000\times$ and printed to a final magnification of $55,000\times$. Many of the fields contained more than one dendrite profile. If one traverse from bundle to bundle failed to yield 12 samples of dendrites, then a second traverse in a trajectory just below the first was made. Another set of samples obtained in a similar manner was obtained by a second investigator in order to test the consistency of the findings obtained with this sampling procedure. Both sets of samples were obtained using 'blind' conditions.

The various dendrite cross-sections and their associated axon terminals were then entered into the computer as described for the light microscopic determination of neuronal size. The data were expressed in the form of distribution curves for each of the variables examined. Parametric or non-parametric statistical tests were then selected for normal and skewed distributions, respectively. Since the synaptic vesicle density and length of synaptic connection showed a normal curve, the two-tailed Student's *t*-test was used. Dendrite calibre, axon terminal size and the number of axon terminals per dendrite cross-section, on the other hand, showed variable degrees of skewedness in their distributions and required non-parametric statistical tests. The computation of exact probabilities for $R \times C$ contingency tables was chosen to analyze these variables, since it permits a detailed, yet flexible, analysis of a distribution¹¹. Thus, comparisons between control and drug-treated groups can be made for either the entire distribution or for discreet portions of the curve (see Figs. 4 and 5). This approach has distinct advantages for the analysis of synaptic parameters, since changes can theoretically occur within sub-populations of various synaptic components. Statistics such as the Wilcoxon Rank Sum or the Kolmogorov-Smirnov test, which examine a population as a whole, do not give ad-

TABLE I

Comparison of neuronal size and density in striatum of haloperidol-treated rats

A light microscopic evaluation performed on $1\mu\text{m}$ thick plastic-embedded material of striatum from control ($n = 4$) and haloperidol-treated ($n = 4$) rats. The values shown represent the combined means and standard errors of the means for the animals within each group.

	Neuron size (μm^2)	Neuron density (cells/ mm^2)
Control	196.4 ± 0.2 ($n = 40$)	62.0 ± 3.0
Haloperidol-treated	$231.2 \pm 15.1^*$ ($n = 40$)	63.9 ± 2.1

* $P < 0.05$ using two-tailed Student's *t*-test.

equate treatment to the types of changes that might occur in a multicomponent system.

Linear regression analysis and analysis of covariance were performed to assess the relationship between terminal size and vesicle number for the two groups.

RESULTS

The results of the light microscopic evaluation of neuronal density and size in control and haloperidol-treated rat striatum are shown in Table I. While the number of neuron somata per unit volume (density) is nearly identical in the experimental and control groups, the size (volume) of the neurons is slightly but significantly, larger in the drug-treated animals. There were no signs of artifactual tissue swelling in the striatum of animals treated chronically with haloperidol.

TABLE II

Comparison of axo-dendritic synaptic parameters in striatum of haloperidol-treated rats

The data were obtained from electron microscopic samples of axodendritic synapses from striatum of control ($n = 4$) and haloperidol-treated ($n = 4$) animals. The values shown represent the combined means and standard errors of the means for the animals within each group.

	Synaptic vesicle density (per μm^2)	Length of synaptic connection (μm)
Control	185 ± 6 ($n = 110$)	0.59 ± 0.03 ($n = 110$)
Haloperidol-treated	$200 \pm 21^*$ ($n = 110$)	$0.58 \pm 0.02^*$ ($n = 110$)

* Not significant using two-tailed Student's *t*-test.

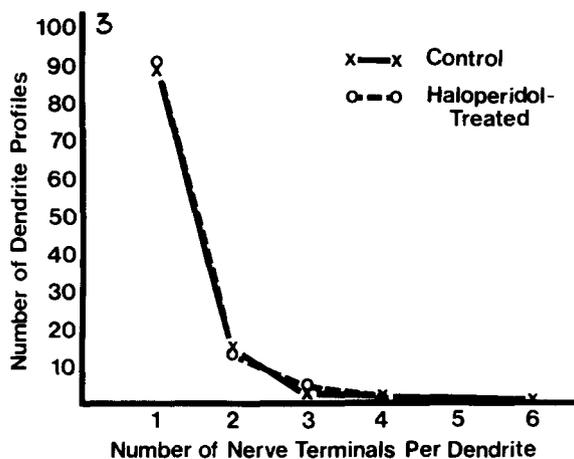


Fig. 3. A distribution curve for the number of axon terminals in rat striatum per dendrite profile for the control ($n = 112$) and haloperidol-treated groups ($n = 114$). Dendrodendritic cross-sectional profiles were sampled as described in the Methods section.

At the electron microscopic level, the number of axon terminals per dendrite in the two groups showed an identical distribution pattern (Fig. 3), as did the length of synaptic connections (Table II). A trend similar to that seen for neuronal size was seen in relation to axon terminal size (Fig. 5) and, to a lesser degree, dendrite calibre (Fig. 4). The peak dis-

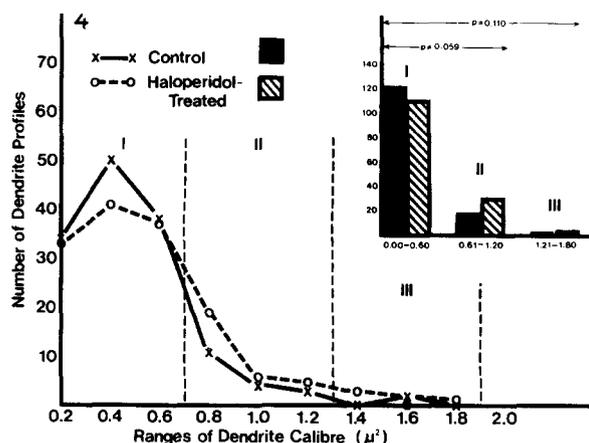


Fig. 4. A distribution curve for the range of dendrite calibres (μ^2) observed among the dendritic profiles which were sampled as described in the Methods section. The curve is divided into 3 ranges as indicated by roman numerals I, II and III. These categories correspond to larger ranges of dendrite calibre less than or equal to 0.6, 1.2 and $1.8 \mu^2$, respectively. These latter 3 ranges of the overall distribution were then subjected to the computation of exact probabilities for a 2×2 (I and II) and a 2×3 (I, II and III) contingency table (see Methods section). The probabilities obtained for each determination are indicated in the histogram shown in the inset in the upper right-hand corner.

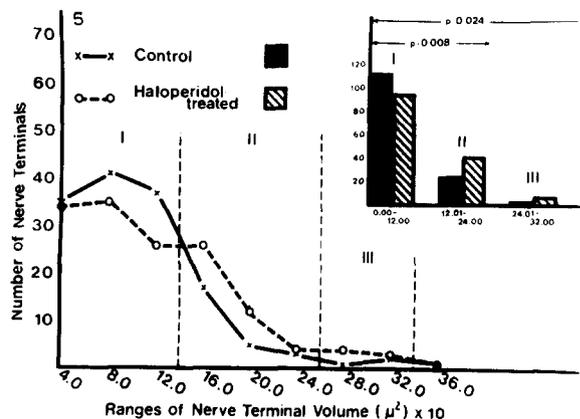


Fig. 5. A distribution curve for the range of axon terminal size (μ^2) which was obtained for the various dendritic cross-sectional profiles for both the control (x) and haloperidol-treated groups (o). The curve is divided into 3 categories as indicated by roman numerals I, II and III. The categories corresponded to the larger ranges of axon terminal size less than or equal to 1.2, 2.0 and $2.4 \mu^2$, respectively. These 3 phases of the overall distribution were then subjected to a computation of exact probabilities for a 2×2 (I and II) and a 2×3 (I, II and III) contingency table (see Methods section). The probabilities obtained for each of these determinations are indicated in the histogram shown in the inset in the upper right-hand corner.

tribution of dendrite calibre occurs in the range of $0.01-0.61 \mu^2$ in control animals and this peak shows some blunting in the drug-treated group. In contrast, there was a trend toward a slightly higher frequency of profiles in the range of $0.6-1.2 \mu^2$, but this shift in the drug-treated group was only significant at $P = 0.059$.

In the case of axon terminal size (Fig. 5), the blunting of the peak distribution in the lower range of size (range I) is more marked and the decrease in the number of terminals occurring in the range $0.4-1.2 \mu^2$ is 22.5%. Paralleling this decrease in the lower range of terminal size, there is a discernible shift in the distribution of terminals toward the range $1.2-2.0 \mu^2$ (range II). Since the number of terminals in this range in control animals is relatively low, the increase in this larger range as a result of the shift in the haloperidol-treated group is 66.6%. The overall number of terminals showing these changes represents approximately 12% of the total population of axon terminals, and this was nonetheless highly significant ($P = 0.008$). The profile of this curve was essentially similar when evaluated for the samples obtained blindly by two different investigators. The fact that the terminal size appeared to be increased in

drug-treated animals (Fig. 5), while synaptic vesicle density remained unchanged (Table II), suggested the possibility that the number of synaptic vesicles may have been increased in these terminals. To assess this possibility further, linear regression analyses were performed for each group using terminal size as the dependent and vesicle number as the independent variable (Fig. 6). The regression lines were similar for both the control ($m = 0.003$, $b = 1.4$, $P < 0.001$) and the haloperidol-treated ($m = 0.004$, $b = 1.6$, $P < 0.001$) groups. An analysis of covariance of this data showed that the number of vesicles behaved as a covariate for the two groups ($P < 0.001$) and that the groups were significantly different ($P = 0.005$) with respect to the two variables.

DISCUSSION

The results of this study support the idea that haloperidol and perhaps other neuroleptic agents can induce structural alterations in the central nervous system. Unlike a previous report¹⁸, no difference in cell number was observed in haloperidol-treated animals. Although the manner in which the data were presented in that report preclude any direct comparisons, differences in experimental design probably account for the discrepancies noted. While in the present study there is only a slight trend towards increased size of neurons and dendrites, the size of axon terminals and their vesicle numbers appear to have been more significantly affected in the haloperidol-treated group. Specifically, the data suggest that approximately 12% of all the terminals may have been enlarged from a median size of $0.8 \mu\text{m}^2$ (range $0.4\text{--}1.2 \mu\text{m}^2$) to a median size of $1.6 \mu\text{m}^2$ (range $1.2\text{--}2.0 \mu\text{m}^2$). Thus, some axon terminals could have effectively doubled in size in the drug-treated group, and might possibly represent a distinct sub-group. Interestingly, there was no change in the density of synaptic vesicles in the haloperidol-treated animals, a finding which is consistent with the observation of a constant linear relationship between terminal size and vesicle numbers. The overall number of synaptic vesicles were, therefore, doubled in some striatal terminals.

It is important to consider whether any of these alterations in synaptic parameters could arise secondarily to artifactual changes in the tissues. The antipsy-

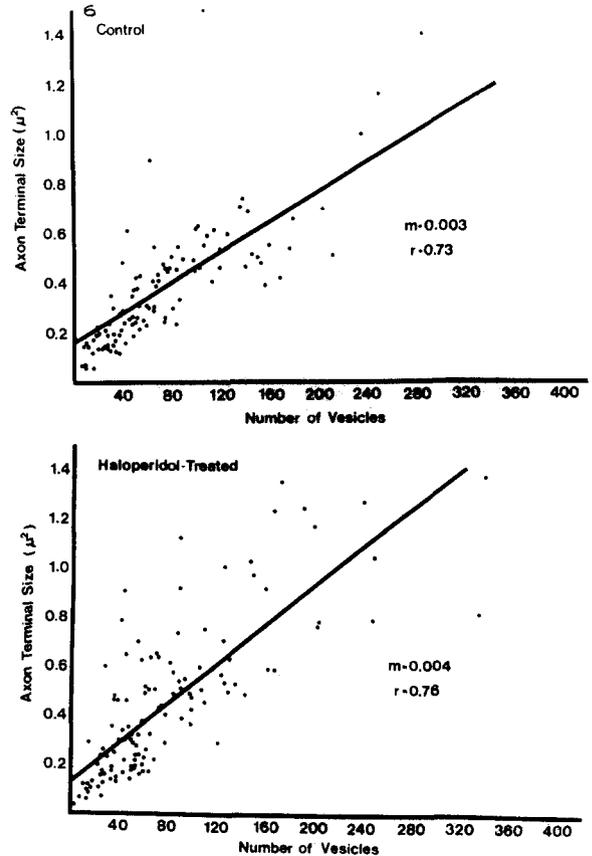


Fig. 6. Linear regression lines obtained with a scattergram plot of points using number of vesicles as the independent and terminal size as the dependent variable. The upper graph shows the control ($n = 110$) and the lower graph the haloperidol-treated ($n = 114$) groups. There were 4 animals in each group.

chotic agents are known to have potent effects on the physicochemical properties of biological membranes. The planar configuration of these heterocyclic molecules permits them to intercalate between fatty acid chains within the lipid bilayer. It is conceivable that such a mechanism could be associated with the preferential occurrence of a fixation artifact arising from a destabilization of the membrane structure. Such a consideration is of particular concern to these current findings since increases of the volume of dendrites and axon terminals could reflect non-specific tissue swelling, a well-known fixation artifact. Two observations argue against this latter possibility, however. First, no morphological evidence of such swelling was seen in either the control or the haloperidol-treated tissues. The most obvious manifestations of fixation-induced swelling would be the appearance of large intracellular vacuoles and areas of

cytoplasm which have lost their characteristic granular appearance. Neither feature was observed in the haloperidol-treated group. The second, and perhaps most important observation that argues against fixation-related swelling having occurred, is the fact that the density of synaptic vesicles in the axon terminals of the haloperidol-treated group was unchanged. Where swelling of cellular compartments occurs, there is a relative volumetric dilution of particles within that compartment. Thus, the density of synaptic vesicles would be expected to decrease significantly in terminals which have swollen to twice their normal size. The fact that this did not occur also argues against the possibility of fixation-related artifact. Another source of artifact to morphometric analyses which is unrelated to fixation is a poorly designed sampling procedure. This potential pitfall was excluded as a serious problem by the use of replicate sampling which was performed by two investigators under 'blind conditions'. As a result of this procedure, it was found that no differences in either the light or electron microscopic results occurred for the respective groups. In addition, the results obtained for individual animals within each experimental group were generally similar, thus excluding a final possibility that the sub-population of an axon terminal which was of a larger size occurred idiosyncratically in only one animal.

The finding of a modest degree of structural change in synapses of the striatum in relation to haloperidol treatment may have some parallels in a variety of biochemical and pharmacologic changes which have been reported for striatum of neuroleptic-treated animals. Both homovanillic acid¹ and 3,4-dihydroxyphenylacetic acid, DOPAC^{7,17}, are increased in the striatum of rats treated both acutely and chronically with haloperidol. These accumulations of dopamine metabolites have been interpreted as evidence of its increased turnover, which may in part be due to an activation of tyrosine hydroxylase kinetics^{12,13,16,22,23} through changes in the feedback loop between the striatum and substantia nigra¹⁴.

Haloperidol also causes an acute increase of acetylcholine turnover in striatum, but this latter effect is not observed after chronic administration of the drug¹⁰. It is unlikely, therefore, that the structural changes which persisted up to 16 weeks of haloperidol administration in this present study reflect altera-

tions in acetylcholine turnover. Since the cataleptogenic effect of haloperidol has been shown to develop tolerance within 2–3 weeks of chronic administration⁹, the pharmacologic changes in acetylcholine turnover which have been reported could reflect both the development and habituation of this extrapyramidal side-effect¹⁰. Information regarding striatal levels of other putative neurotransmitters such as glutamate, enkephalin or substance P in relation to haloperidol is currently not available. At present, therefore, the best known pharmacologic effect of haloperidol within the striatum which persists with chronic administration is the increased turnover of dopamine.

It is tempting to speculate that the occurrence of a shift in the distribution of axon terminals from a median size of $0.8 \mu\text{m}^2$ to $1.6 \mu\text{m}^2$ with concomitant increases in synaptic vesicles could represent a morphological equivalent to enhanced dopamine turnover. In an earlier study, increased numbers of synaptic vesicles were noted with stimulation of the frog pectoralis nerve⁴. This latter effect was attenuated by N-naphthylvinylpyridinium bromide, an agent which inhibits choline acetyltransferase. Those data showed, therefore, that in peripheral nerve both electrical stimulation and transmitter metabolism can affect the number of synaptic vesicles which occur in an axon terminal. Haloperidol which is known to increase both the firing of nigral dopamine cells⁸ and the specific activity of striatal tyrosine hydroxylase could potentially affect the number of vesicles in dopamine terminals. Although no change in synaptic vesicle density (number per μm^2) was observed in this current study, there was an increase in the overall number of vesicles in the group of terminals which appeared to have been enlarged. Thus, the known physiological and pharmacological effects of haloperidol on the nigrostriatal dopamine projection may be reflected in these observed morphological changes. It is not possible at the present time to determine whether those terminals which have undergone these changes correspond to the dopamine innervation. It is of interest, though, that two studies have estimated the proportion of striatal dopamine terminals to be approximately 9%²⁰ and 16%¹⁵, values which compare favorably with the 12% of terminals showing enlargement in this study. It is conceivable, however, that other non-dopaminergic ter-

minals within the striatum could undergo structural alterations which occur postsynaptically in response to the blockade of dopamine receptors. For example, tyrosine hydroxylase immunoreactive terminals have been noted to engage in axo-axonal synapses with cortical afferents to the striatum⁶. The structural and functional characteristics of these presumed glutaminergic cortical endings could potentially undergo substantial alterations in response to blockade of dopamine receptors. Attempts to associate the increase in terminal size in this current study with a particular morphological subtype of axon terminal is confounded by the fact that a variety of striatal nerve endings have a similar appearance, a feature previously noted by others²⁰. Indeed, the changes found in this study could have occurred in more than one type of nerve ending found in striatum.

The results of this study provide further evidence that haloperidol can induce synaptic alterations in the rat central nervous system, an effect which we first noted in the rat substantia nigra⁵. This agent, however, does not appear to produce the same pattern of synaptic change in all brain regions or cells, since that noted in the striatum is strikingly different from the change found in nigra⁵. In striatum, no change in the number of axon terminals per dendrite was observed, while this variable in the substantia nigra showed a significant increase. Thus, the different effects noted in striatum and substantia nigra support the idea that the morphological changes induced by haloperidol may reflect alterations in region-specific

patterns of integration. It is of interest to know whether another brain region which, like the striatum, receives a major dopamine projection would also show a pattern of synaptic alteration similar to that seen in the striatum. The medial prefrontal cortex of the rat is richly innervated by the ventral tegmental A10 dopamine cells. Studies are currently in progress which will determine whether haloperidol can induce structural changes in cortical synapses as well. This latter question is of obvious theoretical importance to our understanding of the antipsychotic agents, since their clinically useful effect on psychosis is likely to be mediated, at least in part, through the cortex. The complexity of the cortex and the fact that it receives a dopamine input with pharmacological properties which are different from those of the striatum^{2,3} could result in a pattern of synaptic change being induced by haloperidol, which is also different from that observed in striatum.

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