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THE TERMINATION OF FIBRES FROM THE CEREBRAL CORTEX AND THALAMUS UPON DENDRITIC SPINES IN THE CAUDATE NUCLEUS: A STUDY WITH THE GOLGI METHOD

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[Plate 76]

The termination of fibres from the cerebral cortex and thalamus upon the dendritic spines of the medium spiny cell of the caudate nucleus has been studied with the Golgi method. Lesions were placed in the cerebral cortex, thalamus or cerebral cortex and thalamus of adult cats and kittens. After survival periods of between 6 and 52 weeks the animals were perfused with a mixture of formaldehyde and glutaraldehyde and the caudate nuclei impregnated by a Golgi technique. The distribution of spines along the dendrites of the medium spiny cell was determined in normal material by counting them over 20 μm lengths of the dendrites, and was compared with their number and distribution after the various lesions. The density of spines on the dendrites varies with the distance from the cell body. The first 20 μm length of dendrite is spine free, but thereafter the number increases to a peak between 60 and 80 μm from the cell body after which the number per 20 μm length decreases. The distribution pattern does not alter after any of the lesions, although the overall number of spines decreases. The decrease after lesions in the cerebral cortex or thalamus is the same, and after a combined lesion of thalamus and cortex is twice as great indicating that the fibres from both these regions end upon spines of the same cells. Statistical analysis shows that these results are significant.

Introduction

Examination of the caudate nucleus with the electron microscope after experimental interruption of the major afferent pathways has yielded information on the mode of termination of the fibres from the cerebral cortex, thalamus and midbrain (Kemp 1968*a*; Kemp & Powell 1971*b*). It has been found that the termination of all of these fibres is similar in many ways, for all end in terminals with asymmetrical membrane thickenings on dendritic spines and shafts of dendrites, though axons from the cerebral cortex and thalamus also end on cell somata. It has been not possible to determine whether these fibres end upon the same regions of the dendritic tree of different cells or upon different parts of the dendrites of the same neurons; it is, of course, possible that there is no difference in the site of termination. This failure to distinguish, with the electron microscope, the region of influence of the different afferent fibres is due to the fact that the sections are so thin that the profiles receiving the degenerating terminals cannot easily be related to a precise region on the dendritic tree nor to the process of a particular cell. Though the size of the dendrite is some indication of its distance from the cell body, the diameter appears to vary little over the major part of its length; a detailed quantitative study would therefore be required to reveal any difference in the diameter of the dendrite and also to show that a particular size of dendrite was related to any one group of afferent fibres. A further

difficulty arises in relation to axospinous contacts as it is relatively rare to find a degenerating terminal onto a spine in continuity with the parent dendrite.

Recent studies have shown that interruption of an afferent pathway may result in decreased impregnation with the Golgi method of the whole neuron (Matthews & Powell 1962; Powell 1967), the peripheral parts of the dendrites (Jones & Thomas 1962; Coleman & Riesen 1968) or the dendritic spines (Globus & Scheibel 1967; Valverde 1967). In view of this failure of impregnation experiments were performed in a series of cats in an attempt to compare the regions of termination of the two major afferents to the caudate nucleus, those from the cerebral cortex and thalamus (Kemp 1970). The dendrites of the caudate nucleus which are most densely covered with spines arise from one cell type, which comprises over 95 % of the total (Kemp 1968*b*), while the dendrites of the remaining cells are relatively spine free. This investigation was therefore limited to a comparison of the distribution of dendritic spines on the one cell variety.

Material and methods

Sixteen adult cats were used in this study but as transneuronal degeneration occurs more rapidly in young animals lesions were also placed in three 7-week-old kittens. Lesions were made in the cerebral cortex by suction and in the thalamus using a stereotaxic method; the thalamic lesions were placed so as to interrupt most of the thalamo-striate fibres but sufficiently far posteriorly to avoid interference with the blood supply to the striatum (cf. Kemp & Powell 1971*b*). Combined lesions of the cerebral cortex and thalamus were placed in some animals. The adult animals were allowed to survive for periods ranging from 13 to 52 weeks and the kittens from 6 to 29 weeks. The animals were perfused under hypothermia with a mixture of 4 % formaldehyde and 1 % glutaraldehyde. After the lesions in the cerebral cortex or after the combined lesions of cortex and thalamus blocks of between 1 and 2 mm thick were taken from the part of the caudate nucleus which is known to receive fibres from the damaged area of the cerebral cortex (Webster 1965). In brains in which a lesion had been placed in the thalamus blocks of the entire cross-sectional area, and of 1 to 2 mm thick, were taken from the whole of the antero-posterior extent of the head of the caudate nucleus. Blocks were also taken from the caudate nucleus of the normal sides of these experimental brains. The material was impregnated by the Golgi–Kopsch method (Colonnier 1964), embedded in L.V.N. and sections cut at 100 μm. Material from normal brains impregnated by the same method was also available for study. The sections were mounted under coverslips using a neutral mountant, and were examined at different magnifications in order to select cells for counts of their spines. The number of spines along the length of the dendrites was counted in units of 20 μm, starting at the cell body; the counts were done using a 100 × oil-immersion objective at a magnification of 1000 and the 20 μm lengths of the dendrites were measured with a micrometer eyepiece. Only neurons which were evenly impregnated and whose dendrites extended reasonably horizontally through the sections were chosen for counting. The ideal situation of a dendrite being placed entirely within a single plane of focus could rarely, if ever, be achieved with this type of cell which is stellate; some counting error was always present therefore as the 20 μm lengths of dendrite could not always be measured accurately and the estimate of spine numbers may be a little high. However, the same error applied to both normal and experimental material and so comparison of the results is considered to be valid. The number of spines was counted on a minimum of three dendrites for each cell, and the values for corresponding 20 μm lengths

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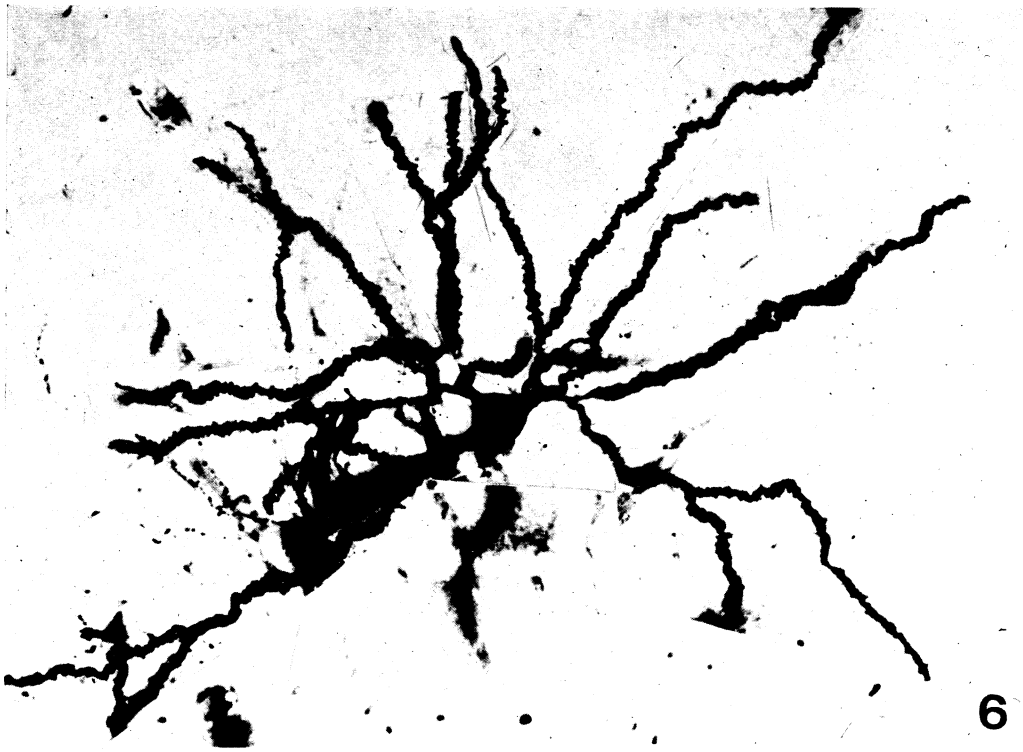

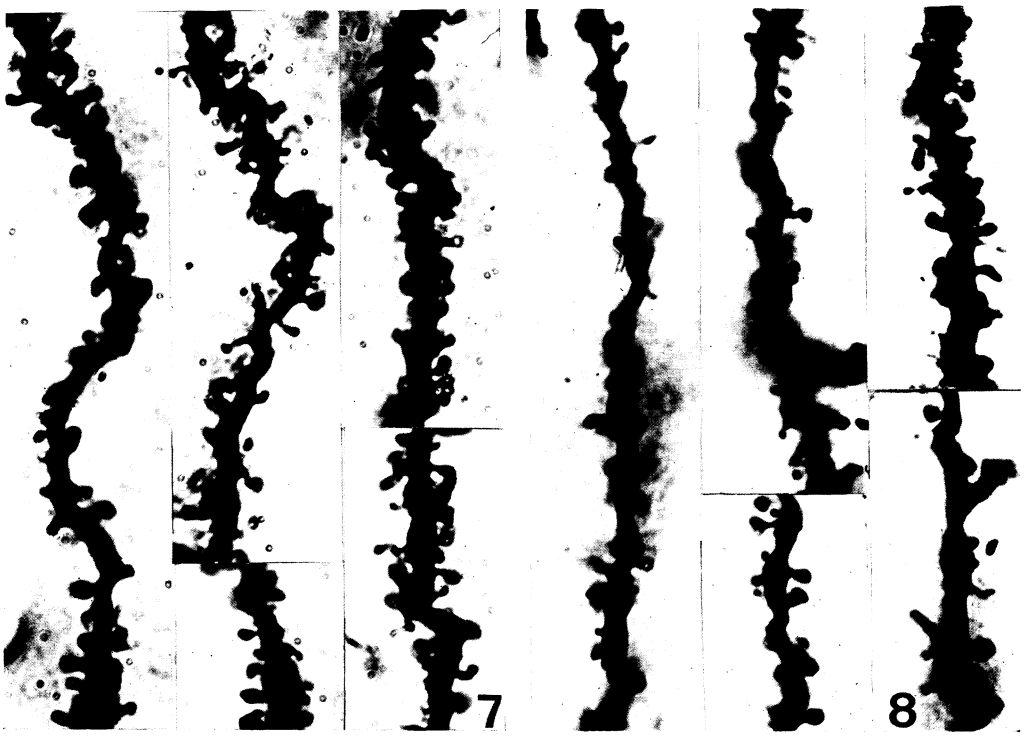

All photomicrographs are of material from the caudate nucleus of the cat impregnated with the Golgi–Kopsch method.

FIGURE 6. Composite photomicrograph, taken at four focal planes, of a medium spiny cell of the caudate nucleus. × 400.

FIGURE 7. Segments from the central parts of the dendrite of the medium spiny cell in the *normal* caudate nucleus. × 2500.

FIGURE 8. Segments from the central parts of the dendrite of the medium spiny cell 100 days *after a combined lesion* of the cerebral cortex and thalamus. × 2500. Figures 7 and 8 are photomicrographs of cells from the normal and operated sides of the same brain.

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of dendrite summed and the mean calculated. Most dendrites reached a length of 140 μm or more and dendrites of less than this length were not included. The variations in average spine number for each cell in the normal and different experimental situations was shown by summing the spine number per 20 μm unit over the first 140 μm length of dendrite for each cell and dividing this by the number of units summed; this gave the mean value per cell. A histogram of the number of cells against the mean value for the spine number was then constructed.

The information gained from this treatment of the results is limited. In order to compare the variations in spine number over each unit length along the dendrite in the various situations, graphs showing the distribution of spines along the dendrite in the normal and each experimental condition were drawn after calculation of the mean spine count for each unit of dendrite in the different groups. A statistical analysis was performed to show whether any apparent differences observed between the normal and experimental groups were valid, and the details of the analysis will be given with the relevant results.

Results

The dendritic spines of the medium spiny cell in the normal caudate nucleus (figures 6 and 7, plate 76) are not distributed uniformly along the dendrites but show a characteristic variation in density depending on the distance from the cell body. In most cases the first 20 μm of the dendrite, closest to the cell body, is free of spines but in a few cases one or two are present; as the number is on average less than 1 this first 20 μm has been considered as spine free for the purposes of the statistical analysis. Beyond this region the number of spines increases rapidly to a peak value of about 27 spines per 20 μm between 60 and 80 μm from the cell soma and then decreases slowly to about 16 spines per 20 μm between 180 and 200 μm from the cell body (figure 2). This pattern is common to all dendrites of this type of cell whether they branch or not, and the pattern of spine distribution on each branch is dependent only on the distance from the cell body.

After lesions in the cerebral cortex or thalamus the cells of the caudate nucleus do not appear to differ in the impregnation of their cell bodies and dendrites from those in normal brains. The proportion of the dendrites reaching lengths longer than 140 μm is unaltered and there is no apparent difference in the diameter of the dendrites. However, the density of the dendritic spines along the dendrites in these two situations is decreased. The cells in the caudate nuclei on the side of the lesion of the two brains studied after combined lesions of the cerebral cortex and thalamus seem less well impregnated as the dendrites are shorter and thinner, and the loss of dendritic spines is more noticeable (figure 8, plate 76). A number of the spines remain, however, but they have smaller heads than in normal caudate nuclei and often shorter stalks and give the appearance of having shrunken. It should be noted that the cells in the caudate nucleus on the opposite side in these two brains are well impregnated and the lengths of the dendrites comparable to those in the normal. Though no counts were made, the number of cells which were impregnated on the normal and the operated sides did not appear to be different. Histograms of the frequency distribution of spines in which the percentage of the total number of cells counted in each experimental situation plotted against the mean number of spines per 20 μm over the first 140 μm of the dendrite is shown in figure 1. Here the spine numbers on the cells in the normal caudate nuclei are compared with those after cortical, thalamic and combined cortical and thalamic lesions. It can be seen that the peak position in the histograms in

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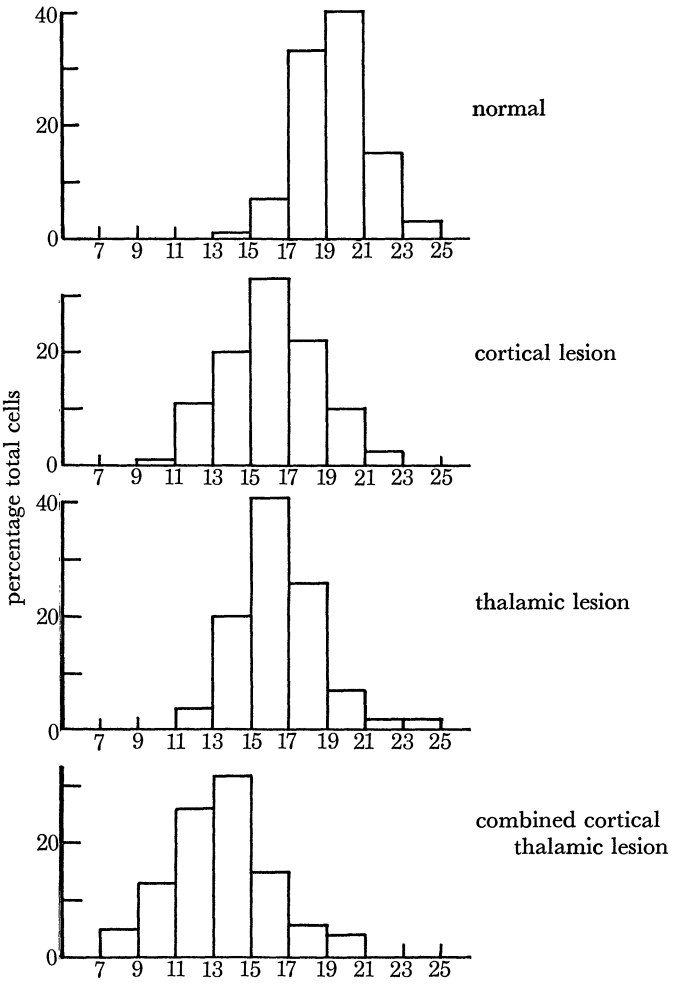

FIGURE 1. Histograms showing the frequency distribution of spines along the dendrites of the medium spiny cells in the normal caudate nucleus and after cortical, thalamic and combined cortical and thalamic lesions.

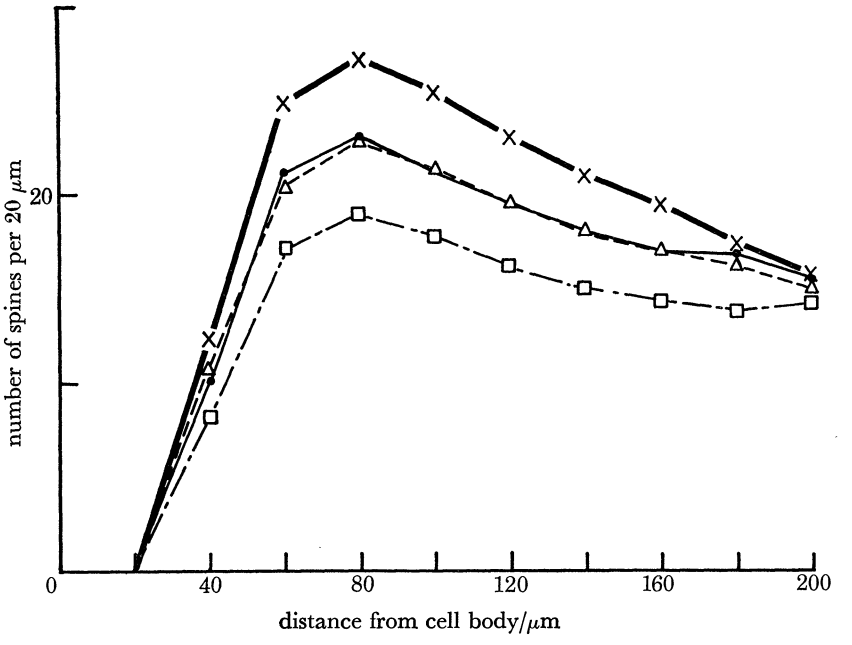

FIGURE 2. Graphs showing the distribution of spines along the dendrites of the medium spiny cells in the normal caudate nucleus (×) and after cortical (△), thalamic (●) and combined cortical and thalamic lesions (□).

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each of the experimental conditions is moved to the left. After lesions in the cerebral cortex and thalamus the mean peak number of spines in each case is 15 to 17 per 20 μm compared with 19 to 21 per 20 μm in the normal. The combined lesion moves the peak position further to the left, to between 13 and 15 per 20 μm suggesting an additive effect of the two lesions.

Comparison of the pattern of the distribution of spines along the dendrite in the different experimental conditions with that in the normal yields further information. After a cortical lesion the distribution of spines along the dendrite is not different from the normal. There is a rapid increase in number between 20 and 60 μm from the cell body, a peak between 60 and 80 μm followed by a gradual decrease in numbers. The curve between 60 and 140 μm from

TABLE 1. NUMBER OF CELLS OF WHICH THE DENDRITIC SPINES WERE COUNTED IN EACH BRAIN

experimental condition	number of cells examined in each brain						total
normal (5 brains)	21	25	21	5	15	—	87
cortical lesion (6 brains)	29	11	22	12	29	20	123
thalamic lesion (6 brains)	26	5	27	15	20	20	113
combined lesion (2 brains)	43	39	—	—	—	—	87

TABLE 2. F VALUES FOR ANALYSIS OF VARIANCE COMPARISON OF TREATMENTS (μm)

	20–40	40–60	60–80	80–100	100–120	120–140	140–160
normal *v.* cortical	1.50	13.54**	52.55***	31.27***	22.83***	9.60*	6.10*
normal *v.* thalamic	2.86	43.43***	29.20***	17.32**	14.00**	9.85*	5.35*
cortical *v.* thalamic	0.02	0.35	0.65	0.10	0.001	0.007	0.0001
cortical and thalamic *v.* thalamic	2.28	25.77**	15.58**	5.76*	5.99*	5.44	3.15

* Significant at $P = 0.05$. ** Significant at $P = 0.01$. *** Significant at $P = 0.001$.

The analysis was not performed for the position 0 to 20 μm as this segment of the dendrite was spine free and for positions beyond 160 μm insufficient data were available.

the origin of the dendrite lies almost parallel with that of the normal but beyond this point it becomes closer suggesting a smaller effect of the cortical lesion in this region; there is also less reduction in the number of spines over the initial 40 μm of the dendrite. The effect of the thalamic lesion is almost identical in lowering the spine number along most of the length of the dendrite with a maximum effect between 60 and 80 μm from the cell body. The distribution of spines after the combined lesion parallels the normal distribution and the decrease in spine number is over the same region of the curve as in the other experimental conditions. The reduction is approximately double that of either of the single lesions along most of the length of the curve. The curves for the spine distribution in the normal and the three experimental situations is shown in figure 2 and the number of brains used in each group and the cells counted in each shown in table 1.

The considerable variation in the survival period of the animals seemed to have no consistent effect on the degree of spine loss; though in one brain the decrease in the number of spines after 13 weeks survival was less than that after 16 weeks, in others the spine loss after 24 weeks was also less than that found after shorter survivals. The age of the animal was also without apparent effect, and it is not possible to say whether the decrease in spine number occurred more rapidly in immature animals as short survival periods were not used in adults. It can be stated, however, that the degree of spine loss was as great in kittens after 7 weeks survival as after 16 weeks in the adult.

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A statistical analysis was performed on these experiments to compare the effects of the different lesions at distances between 0 and 160 μm from the cell body. The tests involved an analysis of variance using a nested design. In all cases the 'between brains' variance in a given treatment group (normal, cortical lesion, thalamic lesion, combined lesion) was significant and this unfortunately increases the effective 'within groups' variances for the different groups; for the comparison of treatments which was required, a pooled estimate of 'within groups' variance was used, because these comparisons when made following a logarithmic transformation (which suitably stabilized the variance) did not produce different results. The results of the analysis of variance for comparison of the treatments is shown in table 2. This shows that the cortical lesion has no significant effect on the spine distribution between 0 and 40 μm from the cell

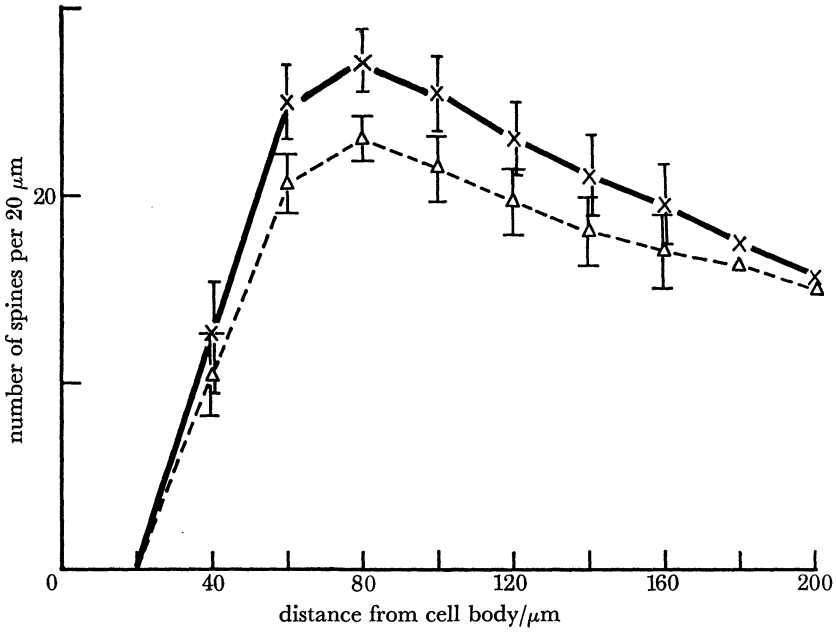

Figure 3. Graphs showing the distribution of spines on the dendrites of the medium spiny cells in the normal caudate nucleus (×) and after lesions in the cerebral cortex (△) with the standard deviations shown by bars.

body but between 40 and 120 μm there is a highly significant influence; beyond this the effect is marginal (figure 3). Comparison of the normal and the thalamic lesion shows an identical pattern (figure 4). Comparison of the spine distribution after cortical and thalamic lesions shows that there is no significant difference in the effects of the two lesions (table 2), and consequently it was not felt necessary to compare the effects of each single lesion with the combined lesion and so it was compared only with the thalamic lesion (figure 5). In this case the F values are only highly significant at 60 and 80 μm from the cell body but with a marginal effect as far as 120 μm along the dendrite.

Comparison of the curves showing the distribution of spines after cortical, thalamic and combined lesions in the two regions suggests that the single lesions have an additive effect. In view of this a factorial analysis was performed. Such an analysis is designed to reveal interactions between different treatments and if no interaction can be shown their effects may be presumed to be independent and additive. The data available showed that the lesions in the cerebral cortex and thalamus had additive effects at all points along the dendrite between 20 and 160 μm from the cell body. The test also shows that the effects of either lesion alone are

significantly different from the effects of the combined lesion over the same part of the dendrite. This factorial analysis is more sensitive and has given results which are stronger than those obtained from the analysis of variance in that the latter showed a significant difference between the single and combined lesions only over restricted portions of the dendrite. The greater sensitivity of the factorial analysis is due to the use of all brains in obtaining a pooled estimate

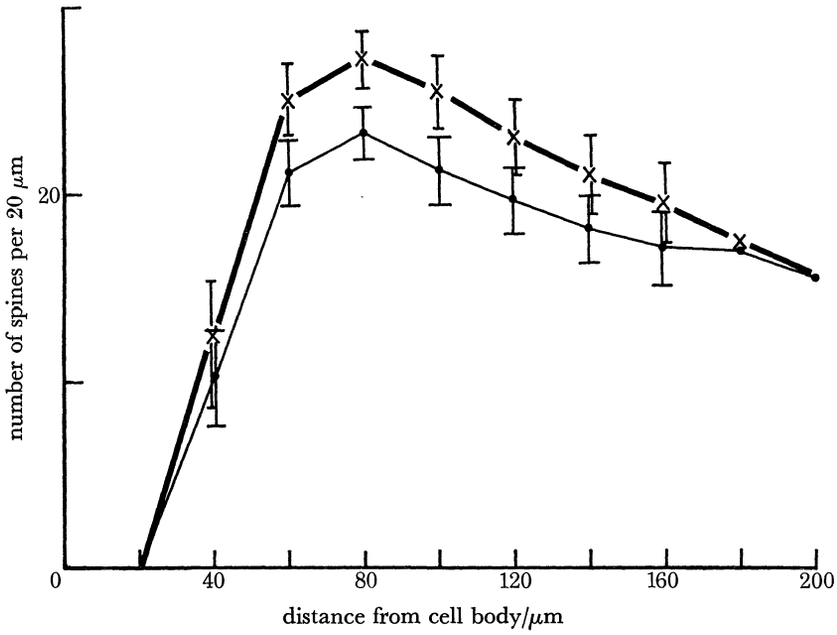

FIGURE 4. Graphs showing the distribution of spines on the dendrites of the medium spiny cells in the normal caudate nucleus (×) and after lesions in the thalamus (●) with the standard deviations shown by bars.

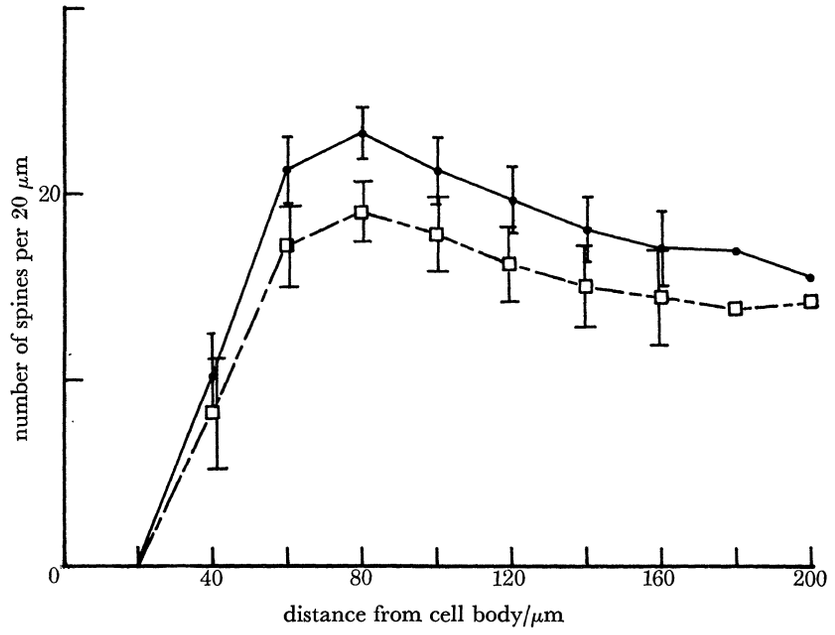

FIGURE 5. Graphs showing the distribution of spines along the dendrites of the medium spiny cells of the caudate nucleus after lesions in the thalamus (●) and combined lesions in the cerebral cortex and thalamus (□) with the standard deviations shown by bars.

of the error. This analysis has thus shown that the effect of the lesions in the cerebral cortex or thalamus are significantly different from the normal over the length of the dendrite between 20 and 160 μm from the cell body, and that the effects of the combined lesion are significantly different from either of the single lesions over the same part of the dendrite.

Discussion

This study of the caudate nucleus provides further evidence that deafferentation of nerve cells gives rise to alterations in their impregnation with the Golgi technique (Jones & Thomas 1962; Matthews & Powell 1962; Globus & Scheibel 1967; Powell 1967; Valverde 1967; Coleman & Riesen 1968; Mouren-Mathieu & Colonnier 1969), and it also shows that this change can occur in a subcortical structure in which the constituent neurons do not exhibit any apparent polarity in the arrangement of their dendrites.

The mechanism for the lack of impregnation is at present not understood. There is evidence from electron microscopic studies that the decrease in spine numbers may be due to actual loss of spines (Szentagothai 1965; Mouren-Mathieu & Colonnier 1969). Deafferentation also leads to failure of impregnation with the Golgi technique of the cells of the olfactory bulb, although these neurons are still present and can be stained with thionin (Matthews & Powell 1962; Powell 1967). A recent electron microscopic study of the rabbit olfactory bulb (Pinching & Powell 1971) shows that transneuronal degeneration, brought about by deafferentation, is manifested by profound changes in the appearance of the cells and their processes; the failure of impregnation with the Golgi method occurs at the same survival time as the first transneuronal alterations seen with the electron microscope. In the rat olfactory bulb Pinching (1969) found that after removal of the olfactory mucosa and degeneration of the olfactory nerve terminals the postsynaptic membrane thickenings, formerly contacted by them, are persistent up to 200 days before the onset of transneuronal changes as shown both by a failure of Golgi impregnation and with the electron microscope. Electron microscopic examination of the caudate nucleus of one of the brains used in the present study (a combined lesion after 112 days survival) showed exposed postsynaptic thickenings, as described by Pinching (1969), but no obvious changes in spines or dendrites were found. Should further examination fail to show alteration in postsynaptic processes it could be interpreted as showing that the failure of impregnation may occur, in the first instance, because of some more subtle change than can at present be detected by the electron microscope; it could be suggested that failure of impregnation may not necessarily indicate primary spine loss, in the same way as failure of impregnation of cells in the olfactory bulb after deafferentation does not indicate loss of cells.

One of the problems encountered in the statistical analysis of the results was the presence of a variance between the brains in each of the treatment groups, including the normal. There are a number of possible reasons for this. It seems unlikely that the differences are due to counting errors for the same errors would occur in each brain, the chief being the difficulty of finding dendrites which lay at a constant depth in the section. A more likely source of error is the different survival times used. However, the longest survival times did not seem to have any consistently greater effect in decreasing the number of spines than the shorter survivals though it was apparent in one or two cases. The other possibility is that there is an inherent difference between the animals even in the normal condition. The statistical analysis used has corrected for this difference between the brains.

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The results indicate that the afferent fibres from the cerebral cortex terminate over the greater part of the dendrites of at least this one cell type but mainly over the region between 40 and 120 μm from the cell body. This pattern of termination is followed exactly by the afferent fibres from the thalamus and the number of fibres terminating axospinously is apparently the same in both cases as there was no significant difference between the spine counts in the two cases at any point along the curve. The fact that the combined lesion of the cerebral cortex and thalamus gave not only the same pattern of spine loss, but gave approximately double the amount of either procedure alone suggests that the afferents are terminating on the same cells and that there are not two populations of medium spiny cells, one receiving cortical and the other thalamic fibres. Furthermore, the figures suggest that most of the fibres terminate on separate spines supporting the finding from electron microscopy that though there are a few spines receiving two terminals with asymmetrical membrane thickenings it is extremely rare to find both degenerating after a combined cortical and thalamic lesion. It appears, therefore, that though there is overlap of the projections from the cortex and the thalamus over the same region of the dendrites of the same cells each afferent influences a separate spine.

Whether or not these figures represent a true quantitative estimate of the input onto spines from either area is difficult to assess in the absence of information on the causes of failure of impregnation of the spines. It may be that spines receiving more than one terminal, even if the second forms a symmetrical synapse, do not show the characteristic loss of impregnation after removal of the extrinsic afferents. In this case the estimate of the number of afferents from a given source terminating on spines would be too small. It is important to emphasize that the method gives no information either on the region of termination or the number of afferents to other parts of the cell.

In the regions of the brain which show a distinct laminar arrangement, such as the cerebral cortex or olfactory bulb, it has been shown that the afferent fibres from different regions of the brain terminate on more or less segregated parts of the dendrite (Lorente de Nó 1934; Blackstad 1956; Raisman, Cowan & Powell 1965; Globus & Scheibel 1967; Valverde 1967; Price & Powell 1970). It is therefore interesting that the homogeneity of the caudate nucleus as seen with the light and electron microscopes is also reflected, but more subtly, in its connexions. Not only do the fibres from the cerebral cortex and thalamus terminate on spines over the same regions of the dendrite but they also influence the same cells.

Some of the spines which remain after a combined cortical and thalamic lesion may receive fibres from a second source which maintains the normal staining properties. This arrangement is possible for it has been shown from examination of the normal caudate nucleus that a small proportion of the spines receive two terminals, one with asymmetrical membrane thickenings and one with symmetrical. The latter is known to be intrinsic and would therefore be undamaged in the combined lesion. Much more rarely both the terminals onto a spine may have asymmetrical membrane thickenings, and the second may arise from afferent fibres to the nucleus or from the intrinsic plexus. Some of the remaining spines will receive only one terminal and this may arise from the midbrain or contralateral cerebral cortex. The number of fibres from these two regions which terminate in the caudate nucleus, however, has been shown to be too small to account for the number of spines remaining, and so these must receive terminals from the collateral axons of the cells of the caudate nucleus.

The distribution of spines remaining after a combined lesion suggests that the terminals of intrinsic axons must be evenly spaced along the dendrites in the same way as the terminals from

the afferent fibres. The fact that the density of spines becomes closer to the normal at the peripheral parts of the dendrite suggests that the intrinsic fibres may predominate in these regions. This is only a tentative suggestion for the number of dendrites which reached lengths greater than 160 μm was comparatively low, and the shape of the distribution curves in this region is therefore based on a much smaller sample than the earlier parts of the curves.

A certain degree of correlation of the anatomical findings with physiological studies has been possible (see Kemp & Powell 1971 *b*). It would be interesting, however, to have support for the observations made here that the afferent fibres from the cerebral cortex and thalamus terminate on the same cells by recording from cells in the caudate nucleus during stimulation of the cerebral cortex and thalamus. If the anatomical findings are correct the same cell would be influenced by both regions, and furthermore, the simultaneous stimulation of the two regions should give an additive effect. It is unlikely, however, that the effects of simultaneous stimulation would be simply additive to cause double the effect as it is known that more fibres from the cerebral cortex end on dendrites.

The suggestion made in a previous paper (Kemp & Powell 1971 *a*) that the dendritic spines and dendrites could act as separate integrating units can now be extended. Diamond, Gray & Yasargil (1970) have proposed 'that the dendritic spine provides a postsynaptic region which is effectively isolated from other synapses in the neurone, in such a way that the immediate and the long-term effects of presynaptic activity at the spine occur with little or no interference from synaptic activity generated elsewhere in the cell'. If this is so, the axon terminals of fibres from the cerebral cortex and thalamus, although ending upon spines closely adjoining each other, could yet have quite separate influences, *both* immediate *and* long-term, upon them. As it is not uncommon to find more than one synapse upon a spine, either two with asymmetrical membrane thickenings or one asymmetrical and the other with symmetrical thickenings, the dendritic spine will be the most peripheral site of integration on the neuron, and at least in the case of one of the synapses having an asymmetrical membrane thickening and the other symmetrical thickenings it can be stated that such integration could be between an extrinsic afferent fibre and an intrinsic axon from a neuron of the caudate nucleus itself. The next level of integration would be a unit length of dendrite which receives terminals onto the spines and dendrites from each of the afferent sources as well as from the intrinsic cells. Further modification of this integrated information can occur at the main stem dendrites and cell body as the main afferent pathways certainly terminate here as well as intrinsic fibres. Final modification of the output of the cell occurs at the initial segment where only intrinsic terminals are found, but it may be noted that although only intrinsic terminals with symmetrical membrane thickenings synapse here two types of such terminals can be distinguished by the size of their vesicles.

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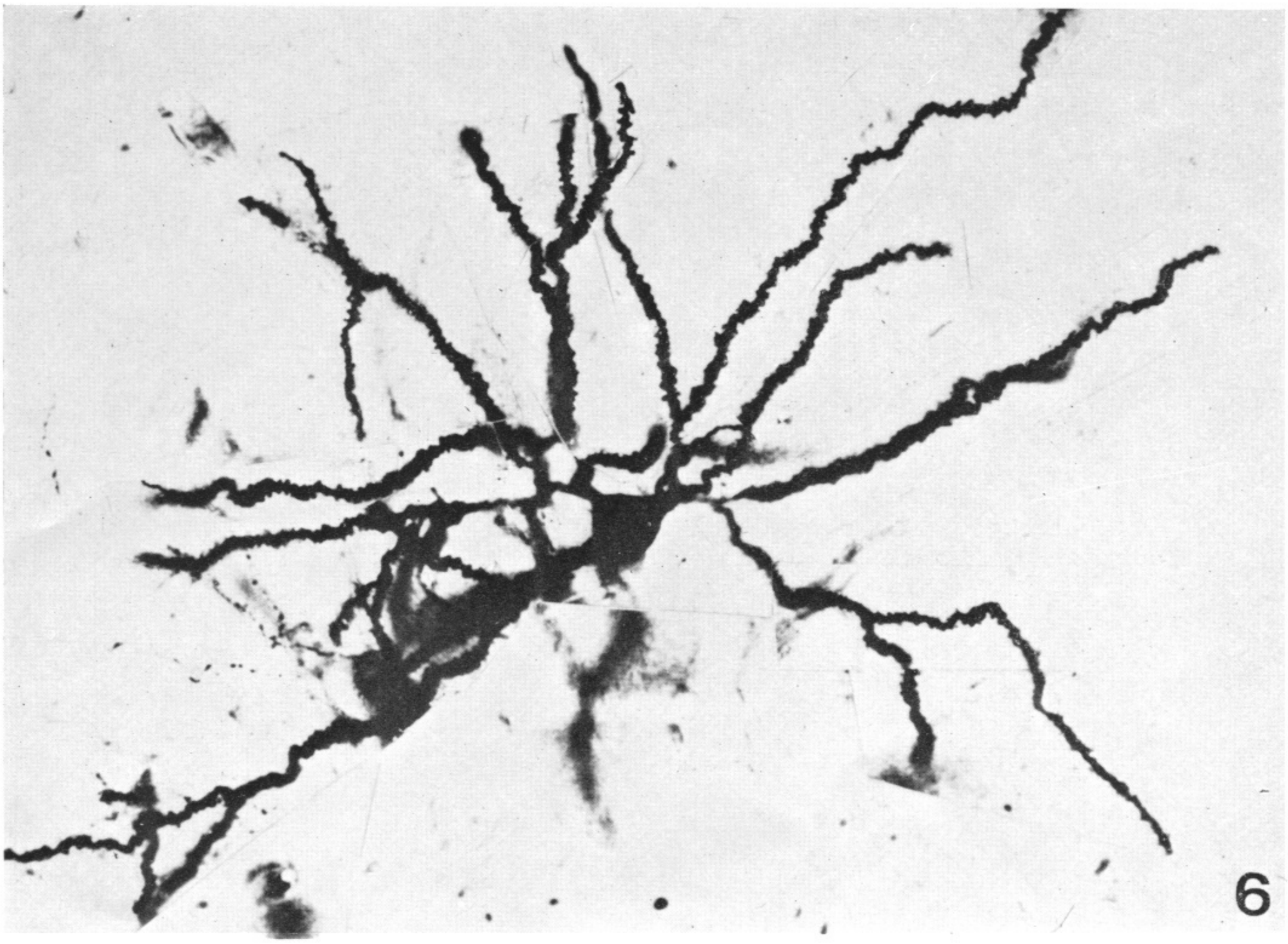
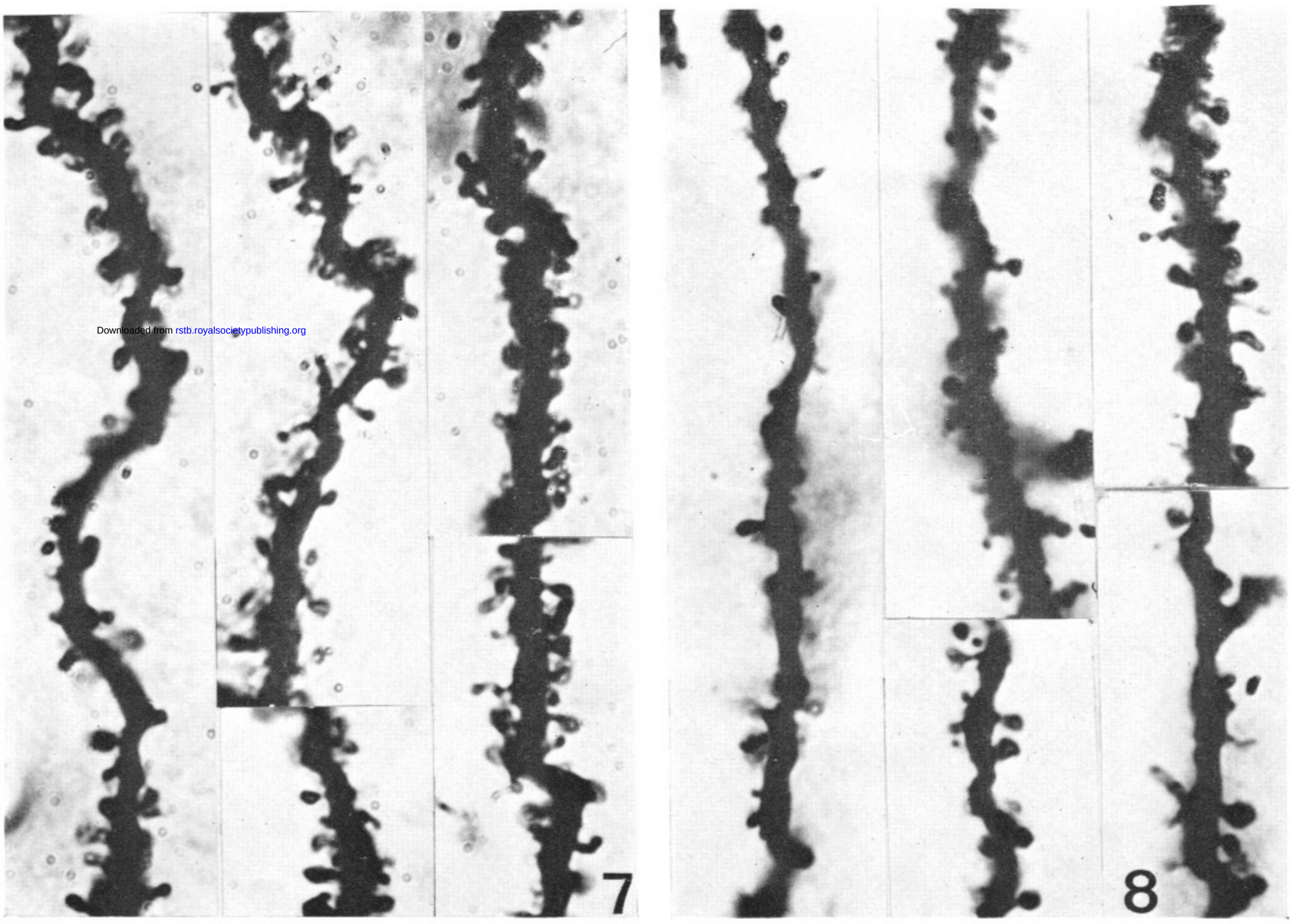
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES





All photomicrographs are of material from the caudate nucleus of the cat impregnated with the Golgi–Kopsch method.

FIGURE 6. Composite photomicrograph, taken at four focal planes, of a medium spiny cell of the caudate nucleus. × 400.

FIGURE 7. Segments from the central parts of the dendrite of the medium spiny cell in the *normal* caudate nucleus. × 2500.

FIGURE 8. Segments from the central parts of the dendrite of the medium spiny cell 100 days *after a combined lesion* of the cerebral cortex and thalamus. × 2500. Figures 7 and 8 are photomicrographs of cells from the normal and operated sides of the same brain.