Pyramidal Cells with Different Densities of Dendritic Spines in the Cortex of the Mouse

Almut Schüz

Max-Planck-Institut für biologische Kybernetik, Tübingen

Neighbouring pyramidal cells in the mouse cortex sometimes have different densities of dentritic spines. This was shown by a quantitative analysis of 10 neurons. For this, a method was worked out which corrects for the spines hidden behind (and in front of) the dendrite. The main result is that there is a positive correlation between the spine densities on different parts of the dendritic tree of one neuron. The possible functional meaning of these findings is briefly discussed.

Introduction

The dendritic surface of many nerve cells in the central nervous system is studded with numerous pinlike processes [1-3]. Especially rich in "spines" are the dendrites of the Purkinje cells in the cerebellum and the pyramidal cells in the cerebral cortex.

Recently, there has been a revival of interest in dendritic spines since it was shown that they are the site of synaptic connections [4-6] and that their development can be influenced by surgical destruction of sensory input [7,8]. Even abnormal environments have their effect on the number and shape of spines [9-12]. Also, abnormal spines have been described in human pathology [13-15].

This paper proceeds from the observation that sometimes neighbouring pyramidal cells of the cerebral cortex have different densities of spines. Since this fact may be related to neuronal learning theories a more quantitative analysis seemed indicated.

Pyramidal cells represent the main cell type of the cerebral cortex. The characteristics of a pyramidal cell are the following [16]: 1. an apical dendrite with a ramification in the first layer, independently of the position of the cell body which can vary between layer II and VI; 2. basal dendrites which radiate from the cell body; 3. an axon which leaves the cortex after taking a straight vertical course toward the white matter; 4. axon collaterals which stay in the cortex.

The following questions were raised:

1. Does the observed difference in "spininess" hold up to statistical analysis?

2. Is there a correlation between the spine densities in different parts of the dendritic tree of a neuron?

Methods

Observations were made on 100 μm thick frontal sections of the brains of adult white mice stained by the Golgi-method (potassium-dichromate-glutaraldehyde-modification by Colonnier [17]). The middle third of the cerebrum (between the frontal and occipital poles) was used because there the plane of the section is oriented parallel to the main-axis of the pyramidal cells. Pyramidal cells in the ventral third of the brain were not considered because of their different morphology.

In the light microscope the cortex was investigated for areas where two pyramidal cells situated close together seemed to differ in their numbers of spines. In five such pairs of cells from three mice the number of spines, the thickness of the dendrites and the length of the spines were measured. The cell bodies of each pair were situated at the same level of the cortex, ranging from layer II to layer V, and their dendritic fields overlapped or were at least contiguous.

For each cell 8 to 11 dendritic segments of 20 to 40 μm length were drawn with the Camera lucida (Fig. 1). These segments were distributed
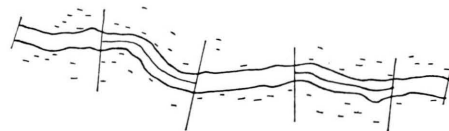

Fig. 1. Segment of 29 μm of an apical dendrite drawn with the Camera lucida ($\times$ 3,000). The points indicate the tips of the spines. In the two regions between the long vertical lines their average projected distance from the axis of the dendrite was measured and the average radius of the dendrite subtracted. The average radius and the number of spines were taken from the whole segment. (For the purpose of this illustration the drawing was reduced to a magnification of $\times$ 2,000.)

on four parts of the dendritic tree: a. branches of the apical dendrite in the first layer, b. stem of the apical dendrite, c. other branches of the apical dendrite beneath the first layer, d. basal dendrites. The segments closest to the cell body were avoided, on both apical and basal dendrites, since these are practically devoid of spines. Also, in order to obtain comparable values, considering the systematic variation of spine density as a function of the distance from the cell body [18-23], in neighbouring neurons, on the apical dendrite stem the samples were chosen at comparable heights.

Spines of all shapes were counted, including those which were partly covered by the dendrite. No attempt was made to include the spines which were hidden behind or which were exactly above the dendrite since these cannot be seen accurately for obvious optical reasons. Therefore, the number of spines counted does not depend only on their real number but also on the diameter of the dendrite and the length of the spines. Assuming that the spines are oriented radially on the dendrite (Fig. 2),
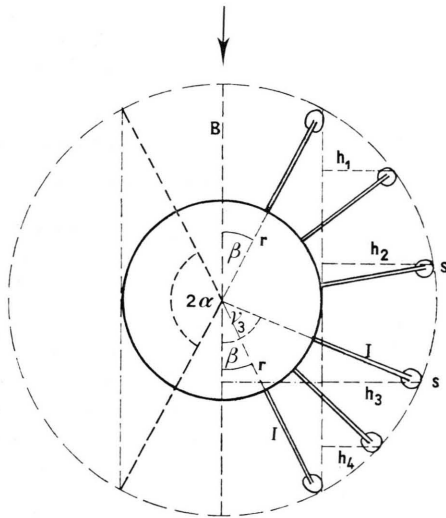

Fig. 2. Scheme of the cross section of a dendrite (circle in the middle) with some spines s. The observer looks in the direction of the arrow. B: optical axis of the microscope; h_{1-4}: lengths of four spines projected onto the visual plane; l, real length of the spines; r, radius of the dendrite; α, angle which subtends one fourth of the spines visible in the microscope; β, angle which subtends one fourth of the spines hidden by the dendrite; γ_3, angle of a spine with the optical axis.

we get the total number N of spines of length l on a piece of dendrite with the radius r from the visible number of spines n:

$$ N = \frac{n \cdot \pi/2}{\arccos \dfrac{r}{r+l}} \, . $$

The radius r of each segment of dendrite was calculated as the average of at least 10 measurements taken from the drawings (Fig. 1). The length l of the spines was calculated from the average length of their visible projections (Fig. 1) on the basis of the following reasoning.

The length l of the spines in general appears shortened on the drawings for two reasons, a. the obliquity with respect to the plane of projection, b. the partial covering of the spines by the dendrite. Very long spines on a very thin dendrite only appear shortened because they are viewed obliquely. On the other hand, the thicker the dendrite is relative to the length of the spines the more b. becomes relevant.

The projected length h_i of a spine of length l is (Fig. 2)

$$ h_i = (l+r)\sin\gamma_i - r \, . $$

To get the projected *average* length $\overline{h}$ of all visible spines of the same length l, the integral between β and $\pi - \beta$ is formed and divided by the angle $\pi - 2\beta$ between the uppermost and the lowest spine which would be just visible:

$$ \overline{h} = \frac{\int_{\beta}^{\pi-\beta} [(r+l)\sin\gamma - r]\,\mathrm{d}\gamma}{\pi - 2\beta} = \frac{\sqrt{l^2 + 2\,l\,r}}{\arccos \dfrac{r}{r+l}} - r \, . $$

With $r = 0$, for an infinitely thin dendrite, we get $\overline{h} = (2/\pi)l$. The other extreme of a thick dendrite with infinitely short spines yields $\overline{h} = (2/3)l$. Surprisingly, the ratio between the average projection $\overline{h}$ of the spines and their true length l is almost the same in the two extreme cases, varying as it does only between $2/3$ and $2/\pi$. It is possible, therefore, to compute the true average length $\overline{l}$ of the spines from their measured average projection $\overline{h}$ independently from their length relative to the thickness of the dendrite.

The correction which has been applied for hidden spines may explain the slight discrepancy between values presented here for spine densities and those of other authors.

Results

Fig. 3 * gives a qualitative impression of the appearance of two neighbouring neurons with different numbers of spines.

In Fig. 4 the spine densities (spines/10 μm of dendrite) of all samples are represented for each of the ten neurons studied. Neighbouring neurons
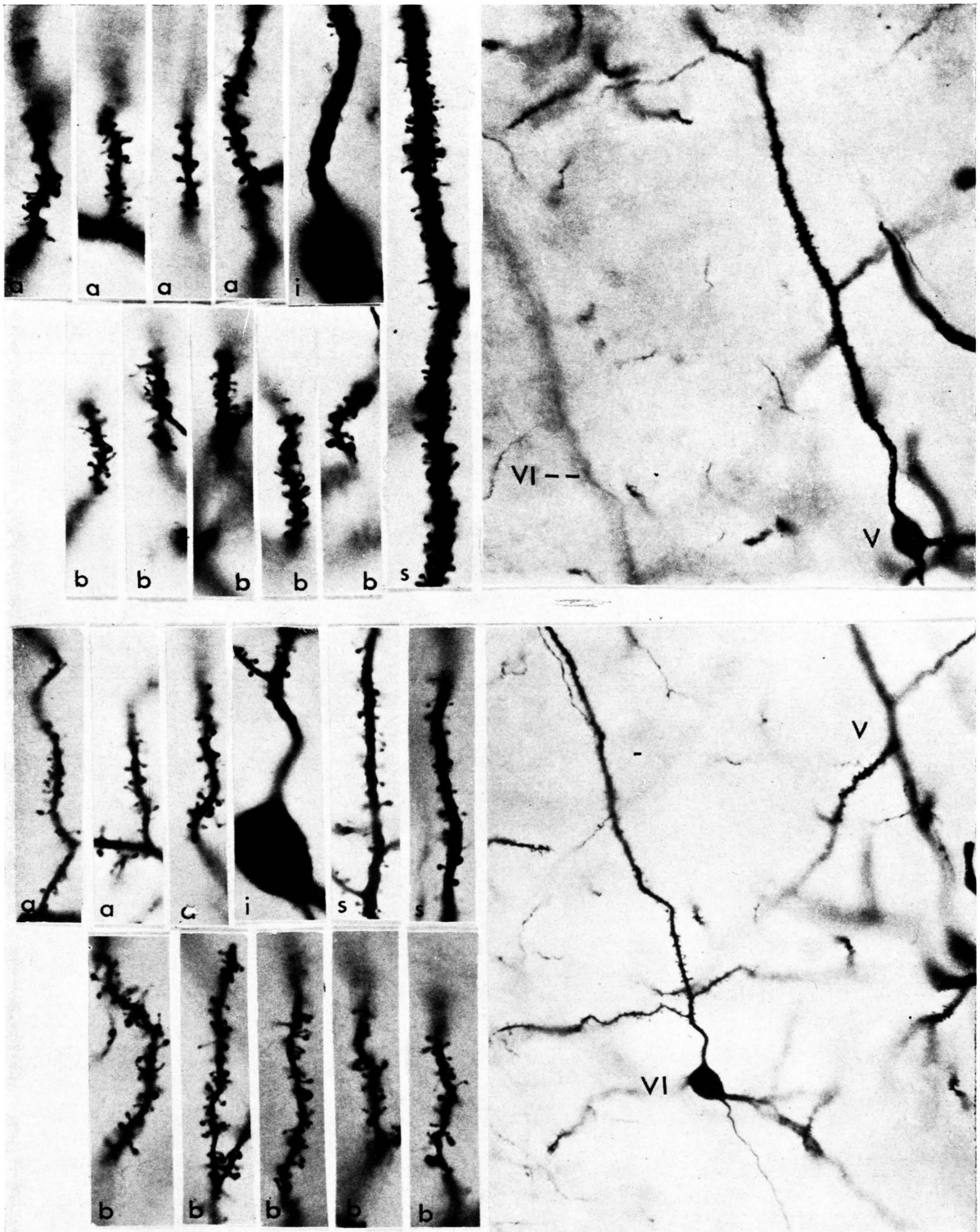
* Fig. 3 see Plate on page 320 a.

Fig. 3. Right side: overview of two neighbouring neurons with different spine densities; above: profusely-spined neuron (V); below: poorly-spined neuron (VI) ($\times$ 400). Left side: enlarged segments of the corresponding neuron on the right side ($\times$ 1000). a, apical dendrite; i, initial part of the apical dendrite; b, basal dendrite; s, stem of the apical dendrite.
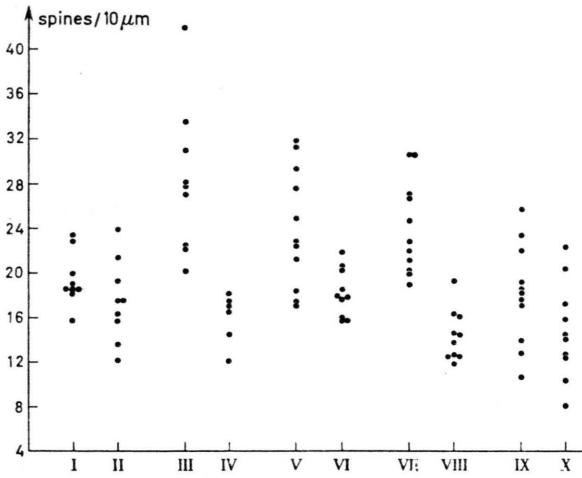
Fig. 4. All measured values of each neuron are arranged one above the other. Each two neurons neighbouring in the preparation are juxtaposed (I/II; III/IV; etc.). Abscissa: ordinal number of the neuron.
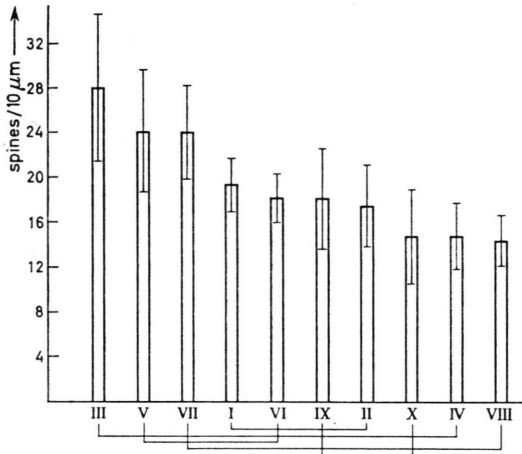

Fig. 5. Mean density of spines and standard deviation in the ten neurons, arranged in order of decreasing density. Neighbouring neurons are connected by a brackett. Abscissa: ordinal number of the neuron.
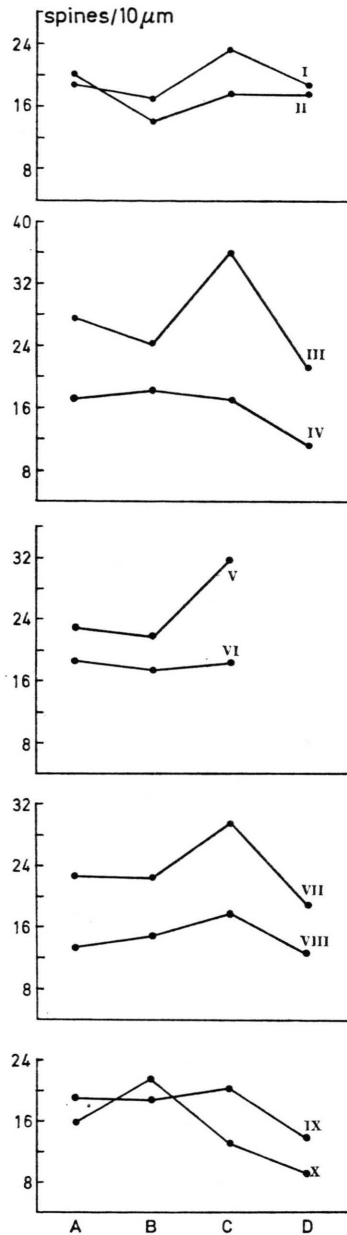

Fig. 6. Each diagram shows a pair of neighbouring neurons. The values of each neuron are lumped into four averages: A basal dendrites, B branches of the apical dendrite beneath the first layer, C stem of the apical dendrite, D branches of the apical dendrite in the first layer.

in the cortex (I and II, III and IV, etc.) are also juxtaposed in the diagram.

Fig. 5 shows the average density of spines of each of the ten neurons in order of decreasing "spininess". There are transitions between the highest and the lowest value which differ by a factor two. The bracketts indicate pairs of neighbouring neurons.

In Fig. 6 for each neuron of a pair the values obtained from all the dendrite segments measured were lumped into four averages: one for the basal dendrites, one for the lower part of the apical

dendrite, one for the stem of the apical dendrite, and one for the branches of the apical dendrite in the first layer. In three of the five cases all values of the profusely-spined neuron are higher than those of the poorly-spined one (III/IV, V/VI,

VII/VIII). They differ significantly (at the 5% level) according to the test of Wilcoxon. The pair of neurons IX/X is a borderline case while neurons I/II don't show a significant difference.

The correlation of the spine densities within one neuron which is apparent in the diagrams becomes more obvious if we take the mean spine count on the apical dendritic tree of each neuron and compare it with the mean value for the basal dendrites of the same neuron (Fig. 7). Neurons rich
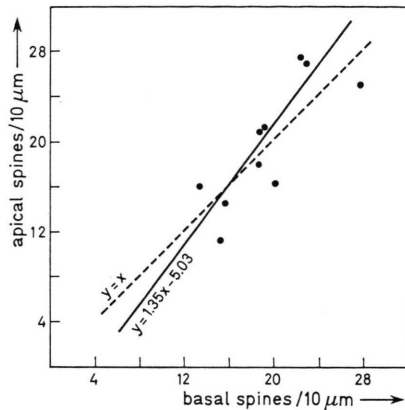

Fig. 7. Relation between the "spininess" of the apical and the basal dendrites. Each point corresponds to the average density of spines on the apical and on the basal dendrites of a neuron. Solid line: regression line.

in spines on their apical dendritic tree are also rich on their basal dendrites. The regression line was obtained by the method of the least squares, the correlation coefficient is 0.90.

Another indication for this correlation is that the values on each neuron show relatively less variation than the values of all neurons together. It is possible to compare the standard deviations of samples with different means when the standard deviation of each sample is normalized by the corresponding mean. This variability coefficient

Table I. Comparison between the variability of the individual neurons and all the neurons together. m, mean value of the spine density; s, standard deviation.

Neuron	Coefficient of variability s/m	Neuron	Coefficient of variability s/m
I	0.07	VI	0.12
II	0.22	VII	0.17
III	0.23	VIII	0.16
IV	0.2	IX	0.25
V	0.22	X	0.29

All neurons taken together: s/m = 0.3.

(Pearson) is, in each case, smaller than that of all the neurons together (Table I).

Discussion

Direct estimation of spine density is treacherous. A quantitative appraisal is necessary and should include corrections for the diameter of the dendrite and the average length of the spines. Only three of the five pairs of neurons chosen because they appeared on inspection to be unequally spiny actually showed a significant difference in the number of spines.

The existence of pyramidal cells with different spine densities is established without any doubt. The differences cannot be attributed to statistical fluctuation because of the strong correlation of the spine densities in different segments of the dendritic tree of one and the same neuron. A possible explanation could be that the neurons with fewer spines are in a pathological condition. Local atrophy of the tissue can be excluded because of the presence, very close together, of spine-rich and spine-poor neurons. Moreover, the neurons which had few spines did not show any pathological symptoms, such as broken up dendrites or shrunken cell bodies etc. Similarly, there was no indication that the small number of spines was due to insufficient staining; in fact, when there was reason to suspect that the dendritic and axonal tree of the neurons was not completely impregnated, the neurons were excluded from the analysis.

We may suspect, then, that the different degrees of spininess depend on different roles of the neurons in the network. Afferences seem to be necessary for the formation of spines [7, 8]. Two facts are important in this connection. Firstly, if two neurons are embedded in the same network of afferents but still differ in their spininess, we may conclude that what makes them spiny is not the general level of afferent excitation, but the detailed afferent constellations which reach them. Secondly, the strong correlation of spininess within one neuron seems to indicate that it is not just a local condition in the afferent excitation, but rather the condition of the neuron as a whole which decides upon the growth of the spines. If the condition for the growth of a spine were defined locally between the activity of an afferent fiber and that of a neighbouring dendrite, a more patchy distribution of spininess could be expected.

Regarding the question of a possible correlation between spine density and dendrite diameter it was found that, in four out of five examples, the more densely-spined neuron was also the thicker one. However, a simple relation between dendrite diameter and the number of spines could not be inferred as is evident in Fig. 8.
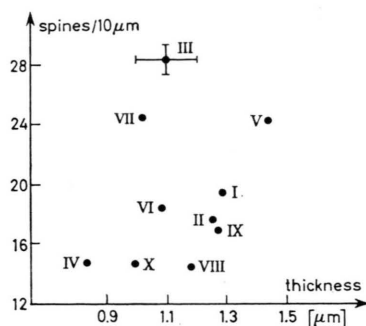
Fig. 8. Spine density *vs* average thickness of the dendrites in the 10 neurons. For neuron III the error of these measurements is shown as a vertical and a horizontal bar, respectively.

[1] Cajal, 1888 and 1891, cited in Cajal: Histologie du système nerveux de l'homme et des vertébrés. Translated by L. Azoulay, Vol. II. Consejo superior de investigaciones cientificas, Instituto Ramon Y Cajal, Madrid 1972.

[2] S. Ramon Y Cajal, Rev. Trim. Microgr. 1, 5—19 [1896].

[3] M. E. Scheibel and A. B. Scheibel, On the nature of dendritic spines. Report of a workshop. Communications in Behavioural Biology, Part A 1, 231—265 [1968].

[4] E. G. Gray, J. Physiol. (London) 145, 25—26 [1959 a].

[5] E. G. Gray, Nature 183, 1592—1593 [1959 b].

[6] E. G. Gray, J. Anat. 93, 420—433 [1959].

[7] A. Globus and A. B. Scheibel, Nature 212, 463—465 [1966].

[8] D. K. Ryugo, R. Ryugo, A. Globus, and H. P. Killackey, Brain Res. 90, 143—146 [1975].

[9] F. Valverde, Exp. Brain Res. 3, 337—352 [1967].

[10] A. Globus and A. B. Scheibel, Exp. Neurol. 19, 331—345 [1967].

[11] S. Schapiro and K. Vukovich, Science 167 [1970].

[12] A. Globus, M. R. Rosenzweig, E. L. Bennett, and M. C. Diamond, J. Comp. Physiol. Psychology 82, 2, 175—181 [1973].

[13] M. Marin-Padilla, Brain Res. 44, 625—629 [1972].

[14] M. Marin-Padilla, Brain Res. 66, 375—391 [1974].

[15] D. P. Purpura, Science 186, 1126—1128 [1974].

[16] V. Braitenberg, J. Theoret. Biol. 46, 421—447 [1974].

[17] M. Colonnier, J. Anat. (Lond.) 98, 3, 327—344 [1964].

[18] A. Globus and A. B. Scheibel, J. Comp. Neurol. 131, 155—172 [1967].

[19] M. Marin-Padilla, J. Comp. Neurol. 131, 475—490 [1967].

[20] M. Marin-Padilla and G. R. Stibitz, Brain Res. 11, 580—592 [1968].

[21] J. M. Mungai, J. Anat. 101, 403—418 [1967].

[22] F. Valverde and A. Ruiz-Marcos, Brain Res. 8, 269—283 [1969].

[23] G. Kunz, W. Kirsche, J. Wenzel, E. Winkelmann, and H. Neumann, Z. mikrosk.-anat. Forsch. (Leipzig) 85, 3, 397—416 [1972].