SYNAPTIC PATTERNS ON DIFFERENT CELL TYPES IN THE DIFFERENT LAMINAE OF THE CAT VISUAL CORTEX. AN ELECTRON MICROSCOPE STUDY

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INTRODUCTION

To adequately describe the circuitry underlying specific cortical activity, it is essential to specify the exact termination of cortical afferents and the detailed connections of intracortical neurons with respect to each other and to the cells whose axons will finally leave the cortical area under consideration.

There can be no doubt that electron microscope studies of degenerating terminals after lesions of afferent pathways or of the cortex itself will be an invaluable tool in this description of cortical circuits.

A systematic study of synaptic patterns on different cell types in the different laminae of the cerebral neocortex is the necessary prerequisite of a useful interpretation of such degeneration experiments. The many published studies on the morphology of normal and degenerating synapses in the cerebral cortex observed in osmium immersed and aldehyde perfused material have not taken into account the heterogeneity of the cortex as manifested by its lamination and the different cell types it contains, except in very general terms.

The area chosen for this preliminary attempt at a finer dissection of synaptic patterns in the neocortex is Otsuka and Hassler's area 17 in the cat.

MATERIAL AND METHODS

The cats were perfused with fresh formalin under artificial respiration and appropriate blocks of cortex were immersed in osmic acid, stained with uranyl acetate in the block and embedded in Araldite. Sections measuring 1–2 mm in width and at least as long as the whole thickness of the cortex were placed on Robertson slit grids and orientated to permit a continuous, unobstructed view of the whole thickness of the cortex from layer I to the white matter. The sections were stained with lead citrate.

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RESULTS

The morphology of cortical synapses in formalin fixed, uranyl acetate and lead citrate stained material

Synapses can be identified in this material as elsewhere in the central nervous system by the apparent thickening of the apposed membranes of two cytoplasmic profiles, one of which contains synaptic vesicles (Figs. 1 and 2). In the cerebral cortex two types of synaptic junctions can be readily distinguished on the basis of the synaptic membrane differentiation.

In the first type of synapse (Fig. 1, synapse a. See also, for example, Fig. 9, synapse No. 24.) both pre- and postsynaptic membranes appear denser and generally more nearly parallel to each other than apposed non-synaptic membranes which tend to be wavy in formalin fixed material. The postsynaptic membrane is bordered on the cytoplasmic side by a dense thick opacity corresponding to De Robertis’s subsynaptic web and Van der Loos’s subsynaptic organelle. This postsynaptic opacity can be seen as different from but continuous with the sharp line of the postsynaptic membrane if the synaptic membranes are cut strictly normal to the plane of section (Fig. 1, synapse a between arrows. See also, for example, Fig. 9, synapse No. 24). The postsynaptic opacity and postsynaptic membrane fuse into a fuzzy continuum with the slightest obliquity of section (Fig. 1, synapse a above arrows). If the whole synaptic contact is cut in this fashion, it cannot be characterized and identified (Fig. 7, synapse a).

In the second type of synapse (Fig. 2. See also Fig. 9, synapse No. 90) the pre- and postsynaptic membranes also appear denser and more nearly parallel to each other than the wavy non-synaptic membrane. The density of the postsynaptic membrane is sometimes thicker than the presynaptic membrane (Fig. 2) but it is not bordered by a dense, compact cytoplasmic opacity seen as different from the membrane, vis-à-vis the vesicle agglomeration. The cytoplasm immediately bordering the postsynaptic membrane is essentially similar to that found within the rest of the postsynaptic profiles, i.e. it is pale and contains small, discrete fluffs of granular, opaque material. Occasionally, a small symmetrical opacity, bordering both pre- and postsynaptic membranes can be seen to the side of the vesicle agglomeration (Fig. 5, arrow. Fig. 9, synapse No. 86, arrow). These opacities are interpreted as a type of symmetrical desmosome joining pre- and postsynaptic membranes. They have also been seen to the side of the first type of synaptic membrane differentiation.

In both types of synapses the extracellular space is sometimes (though not always) wider than that between undifferentiated membranes (Fig. 9, compare synapses No. 38 and 65, both of which belong to the first type). In both types of synapses (Figs. 1, 2 and 5) opaque material can be found in this space but it is not as highly structured as in osmium immersed cortex.

By and large these two types of synapses thus correspond to Gray’s type 1 and 2 as seen in OsO₄ immersed, PTA stained cortex. The analogy is not perfect however because the extracellular space width and opacity characteristics defining Gray’s types do not seem to hold on formalin fixed material. Moreover the distribution of the two
types described herein on different parts of neurons does not correspond exactly to that of Gray's type 1 and 2, as will be obvious under the next heading.

It would therefore be improper to refer to them as Gray's type 1 and 2 and we must be content to refer to the synapses with a cytoplasmic postsynaptic opacity as asymmetrical synapses and to the others as symmetrical synapses. It must be fully realized that the symmetrical synapse is not strictly symmetrical since the postsynaptic membrane is sometimes thicker than the presynaptic membrane (Fig. 2) and since the components within the related cytoplasmic profiles are different. This fault in terminology is more than compensated by its descriptiveness which naturally calls to mind one or the other type.

Gray's distinction of two synaptic types has recently been criticized as representing but two extremes of a single continuum. This criticism is a priori even more applicable to the presently described asymmetrical and symmetrical types, relying as it does on a single criterion: the presence or absence of a variably thick cytoplasmic postsynaptic opacity bordering the postsynaptic membrane. In order to determine whether these two types of synapses should be considered as two extremes of a continuum or as two distinct types, a random series of 'normally cut' synapses has been analyzed. Photomicrographs were obtained of the first 100 synapses to appear on the viewing screen through a large number of vertical penetrations from layer I to layer VI and: (1) whose membranes were cut normal to the plane of section for the whole length of the thickening and (2) where vesicles in the presynaptic profiles were seen to conglomerate towards the thickening. (The membranes were judged to be cut normally

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**Fig. 1.** Synaptic knobs in cat cerebral cortex. Synaptic knob a contains spheroidal vesicles. The synaptic membrane differentiation is of the asymmetrical type (see text). The portion between arrows is cut normal to the plane of section and shows a sharp postsynaptic membrane different from the postsynaptic cytoplasmic opacity. The portion above the arrows is cut obliquely; a sharp postsynaptic membrane cannot be seen. Synaptic knob b contains the flattened type of vesicle population. × 32,500.

**Fig. 2.** Synaptic knob in cat cerebral cortex illustrating the 'symmetrical' type of membrane differentiation as described in the text. The vesicle population is of the flattened type. × 32,500.

**Fig. 3.** Synaptic knob in cat cerebral cortex containing a large number of dense core vesicles. × 32,500.

**Fig. 4.** Synaptic knob in cat cerebral cortex containing a web-like tubular network. × 32,500.

**Fig. 5.** Dendritic spine, identified by a highly structured spine apparatus, with two synaptic contacts. Contact a is of the asymmetrical type. Contact b is of the symmetrical type with flattened vesicles. Arrow indicates symmetrical desmosome-like structure to the side of the vesicle agglomeration. × 32,500.

**Fig. 6.** Dendritic spine, identified by a recognizable spine apparatus, with two synaptic contacts. Synapse a is of the asymmetrical type with spheroidal vesicles. Synapse b is of the symmetrical type with flattened vesicles. × 32,500.

**Fig. 7.** Dendritic spine, identified by a recognizable spine apparatus, with two synaptic contacts. Synapse a is related to spheroidal vesicles. Synapse b is symmetrical and associated with the flattened type of vesicle population. × 32,500.

**Fig. 8.** Dendritic spine, identified by an ill-defined organelle made up of sacs and dense material and interpreted as a spine apparatus. Synapse a is of the asymmetrical type with spheroidal vesicles. Synapse b is of the symmetrical type with flattened vesicles. × 32,500.

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when they were sharp and distinct. This implied that they could be seen as different from the postsynaptic opacity when it was present: vide supra.

It was deemed important to reproduce the whole series (Fig. 9) for the reader to be able to evaluate the validity and limits of the data interpretation, to be able to visually understand the abstract 'literary' concepts derived therefrom and indeed to have the opportunity to disagree with them.

The 100 synapses have been displayed in approximate order of decreasing postsynaptic opacity. From synapse No. 1 to about 56 it was practically impossible to determine this order since they all seemed equivalent. From there on, the postsynaptic opacity diminishes in thickness and/or density up to synapse No. 79, the last which was judged to possess a clearly recognizable compact opacity. Any synaptic contact whose membrane differentiation lies within the spectrum of synapses No. 1–79 will henceforth be classified as an asymmetrical synapse.

No postsynaptic opacity other than the aforementioned discrete, particulate granular fluffs can be seen in the postsynaptic cytoplasm of synapses No. 83–100. Contacts whose morphology is equivalent to these will be treated as symmetrical synapses.

Three synapses (No. 80–82) are difficult to classify. They are considered as a transition zone between synapses whose postsynaptic membrane is bordered by a compact opacity and those bordered by discrete granular material, which remains particulate.

Though each individual observer might not necessarily agree exactly with the limits thus set, it is believed that all would agree that the majority of synapses in this series are easily classified as one or the other type and in this sense at least (see Discussion) the synaptic types do not represent two extremes of a continuum. That they gradually merge into one another seems correct although the abruptness of the transition from one to the other strongly suggests the existence of two types of synaptic membrane differentiation albeit with a certain minimal amount of overlap.

Different types of synapses may also be distinguished on the basis of the types of vesicles found in the presynaptic profiles.

Some terminals contain virtually only circular to slightly oval vesicles whose sizes are sometimes relativey homogeneous (Fig. 1, synapse a), sometimes quite varied (Fig. 9, synapse No. 31). There is a tendency for very small vesicles to be found close to the presynaptic membrane (see, for example, Fig. 9, synapses No. 59 and 37). In a few terminals a distinct row of small vesicles can be seen bordering the presynaptic membrane (Fig. 9, synapses No. 1, 4, 6, 20, 22, 23, 75). Terminals with this type of 'spherical' vesicle population are by far the most common type found in the cerebral cortex.

Other terminals contain an admixture of elongated and small round vesicles (Fig. 1, synapse b). The diameter of most round vesicles is close to the narrow dimension of the elongated vesicles. They are comparable in size to the smallest vesicles of the

Fig. 9. Series of one hundred, randomly selected synapses cut normal to the plane of section. For explanation see text. Arrow of synapse No. 86 indicates a symmetrical desmosome-like structure to the side of the vesicle agglomeration. × 42,000.

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spheroidal population *i.e.* those found close to the presynaptic membrane. The proportion of elongated to circular vesicles is extremely variable. In some extreme cases most vesicles are small and circular and there is only a suggestion of flattened ones (Fig. 7, synapse b). In these instances, the small size of the vesicles still permits the terminals to be clearly distinguished from the type of terminal containing the spheroidal vesicles (Compare synapses a and b in Fig. 7). Vesicle populations similar to that of synapse b in Fig. 7 are classified as 'flattened' vesicles so long as vesicles are seen away from the presynaptic membrane: if they are only seen close to the membrane, it is impossible to decide to which group the vesicle population belongs (Fig. 9, synapse No. 3) since the vesicles of the spheroidal population are small in that location.

In the series of one hundred synapses illustrated in Fig. 9, those terminals which can confidently be said to contain the spheroidal type of vesicle population (see for example synapses No. 1, 5, 6, 11, 13, 17, 22, 31, etc.) are associated with asymmetrical synapses. No clearly identifiable spheroidal population can be seen associated with symmetrical synapses No. 83–100.

Conversely, those terminals which can confidently be said to contain the flattened type of vesicle population are associated with symmetrical synapses (see for example synapses No. 89, 90, 94, 97, 100). No clearly identifiable flattened population is associated with asymmetrical synapses No. 1–79.

There is thus a relatively strong correlation in the cerebral cortex linking asymmetrical synapses to spheroidal vesicles and symmetrical synapses to flattened vesicles. Some flagrant exceptions have been seen in the course of the study but they are rare. This correlation reinforces the concept that two synaptic types do occur in the cortex.

Although one to three large dense core vesicles (Fig. 2, arrow) can sometimes be seen in terminals containing the flattened or the spheroidal type of vesicles, some extremely rare terminals contain a large number of dense core vesicles (Fig. 3).

Another relatively rare type of terminal contains what appears to be a web-like tubular network separated from the presynaptic membrane by synaptic vesicles (Fig. 4).

The latter two types of synaptic knobs have not been extensively studied.

*The distribution of synaptic types in the cerebral cortex*

The majority of neuronal processes in the first layer of the cerebral cortex are the spine loaded bushy terminals of underlying pyramidal cells and recurrent collaterals of axons arising from cells in the immediately underlying deeper layers of the cortex. Axons and beaded dendrites of endogenous cells are much fewer in number.

On electron microscopy, many large pale staining dendrites with spines budding off into the neuropil can be seen in this layer (Figs. 11, 12 and 13). They are interpreted as pyramidal cell dendrites. Synaptic differentiations can usually be seen on their spines. Some rare synapses are also present on the dendritic trunks bearing the spines but large expanses of the dendritic trunks are devoid of contacts. None are present on those illustrated in Figs. 11, 12 and 13.
When the synaptic junctions of spines are cut normal to the plane of section and when the presynaptic profile contains a sufficient number of vesicles for the population to be characterized, it appears that the very great majority of spines are associated with asymmetrical synapses related to spheroidal vesicles (Fig. 13).

More rarely some spines do form synapses of the symmetrical type (synapses labelled b in Figs. 5, 6, 7 and 8). The terminals associated with these symmetrical synapses always contain vesicles of the flattened type. These relatively rare symmetrical contacts with flattened vesicles are often associated with asymmetrical synapses (synapses labelled a in Figs. 5, 6 and 8) with spheroidal vesicles (synapses labelled a in Figs. 6, 7 and 8) on the same spine. Evidence of a similar coupling of two asymmetrical synapses with spheroidal vesicles or of two symmetrical synapses with flattened vesicles on the same spine has not been clearly seen on the material so far examined.

The cytoplasm of the spines but rarely contains the highly structured dense bars and membranous sacs (Fig. 5) labelled 'spine apparatus' by Gray and observed by others in osmium immersed cortex. Schultz and Karlsson have recently underlined the relative absence of the spine apparatus in aldehyde fixed material. However, even on formalin perfused cortex, the spines often contain ill-defined membranous sacs and opaque material (Figs. 6, 7, 8 and 13). If these diffuse organelles are taken to correspond to the more rigidly organized spine apparatus of osmium fixed cortex, it would seem that spine apparatuses are as frequent in aldehyde as in osmium fixed material. They serve as a convenient label for spines when the plane of section severs the spine from its dendritic trunk.

The dendritic trunks bearing these spines (as already mentioned) receive relatively few contacts. Those that are present are either of the asymmetrical type with spheroidal vesicles or of the symmetrical type with flattened vesicles.

The pyramidal cell bodies at the top of layer II are sometimes identified by their triangular shape and the presence of a large apical dendrite ascending towards layer I (Fig. 10). Synaptic contacts are found on these cell bodies (1–6 per soma per section) but wide expanses of the membrane are devoid of contact. Fig. 10 illustrates the upper limits of this range in synapse density. All contacts which could be clearly identified on these cell bodies have so far been only of the symmetrical type with flattened vesicles (Fig. 14).

The initial spine-free portions of the apical dendrites seen arising from the pyramidal cells, like their spine-bearing branches, receive relatively few contacts. The distribution of synaptie types on this part of the apical dendrite is similar to that of the cell body, i.e. all those which could be clearly identified have so far been only of the symmetrical type with flattened vesicles. The same can be said for the equally spine-free parent stems of basal dendrites.

The rare cell bodies in layer I all belong to cells of the stellate type, i.e. cells which are virtually spine-free and without an apical dendrite. On these somata, 3–6 synapses can be seen per cell per section. Some of the synaptic complexes on these cells are of

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Fig. 10. Pyramidal cell body from the second layer of the cat cerebral cortex. Arrows indicate synaptic contacts. × 6,000.

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the symmetrical type with flattened vesicles as the previously described axosomatic contacts on pyramidal cells. Other synapses are of the asymmetrical type with spheroidal vesicles (Fig. 19). The postsynaptic opacity though often thin is quite definite. It also tends to be narrow, covering but a small percentage of the zone of apposition between pre- and postsynaptic profiles. The latter characteristic is more typically associated with Gray's type 2 synapse in OsO4 immersed PTA stained material.

Within the neuropil of layer I, short lengths of dendritic trunks receive rather more synaptic contacts than what has just been described for spine-bearing dendrites (Fig. 17). Some extremely rare examples are studded with contacts (Fig. 16). Dendritic trunks of the type illustrated in Figs. 16 and 17 have never been seen giving rise to spines. They often show swellings reminiscent of stellate beads. Some have been seen in continuity with cell bodies in layer I. They are thus interpreted as stellate cell dendrites.

The contacts found on these stellate cell dendrites are either of the asymmetrical type with spheroidal vesicles or of the symmetrical type with flattened vesicles, when the synapses can be clearly classified as one or the other type. The impression is that a relatively greater number of synapses are difficult to classify on these dendrites. It seems that more of them fall within the transition zone illustrated in Fig. 9, synapses No. 80-82 than is the case for contacts on pyramidal cells.

Many oval cell bodies in layer IV (Fig. 15) also receive both types of synaptic complexes. These oval cell bodies, like many of those in layer I, tend to have a somewhat denser cytoplasm than cells which could be interpreted as pyramidal. Compare Figs. 15 and 10 to see how the general density of the stellate cell perikarya is such that they do not stand out clearly from the surrounding neuropil, while the pyramidal cell cytoplasm is better contrasted against the neuropil density. An axosomatic layer IV asymmetrical contact with spheroidal vesicles similar to that found on layer I stellate cells (Fig. 19) is illustrated in Fig. 20. Some cells receive only 3–6 synaptic contacts per section. Others, fewer in number, had 20 and more synapses so that a large portion of the soma membranes was covered by contacts. The one illustrated in Fig. 15 has approximately 30 (arrows).

In this same layer IV, more spine-free dendrites with many synaptic contacts can be seen than in layer I. It is not as rare to see some with a great density of contacts (Fig. 18). They are again sometimes seen in continuity with oval cell bodies. The relatively greater number of these dendrites in layer IV reinforces the interpretation that they do correspond to stellate cell dendrites. The distribution of synaptic types on these dendrites corresponds to that described for layer I 'stellate' cell dendrites.

Fig. 11. Spine bearing dendritic trunk from the first layer of the cat cerebral cortex. Note presence of synaptic contacts on spines and absence of synaptic differentiation on dendritic trunk. × 11,000.
Fig. 12. Same as Fig. 11. × 11,000.
Fig. 13. Spine bearing dendritic trunk from the first layer of the cat cerebral cortex. Note the spine apparatus and the asymmetrical contact with spheroidal vesicles. × 18,000.
Fig. 14. Symmetrical contact with flattened vesicles on layer II pyramidal cell body. × 27,000.
DISCUSSION

Two types of synaptic membrane differentiations can be seen in formalin fixed cerebral cortex. They are analogous to Gray's type 1 and 2 described in OsO₄ immersed, PTA stained material. The analogy is not perfect because the extracellular characteristics of Gray's types are not constant in formalin fixed material. Again, though the distribution of the two synaptic types on different parts of neurons corresponds remarkably well to that of Gray's types, it is not rigorously the same. The symmetrical type here described is found, though rarely, on dendritic spines while Gray's type 2 is absent from these sites. The asymmetrical type though absent on pyramidal cell bodies is characteristically present on what has been interpreted as stellate cell perikarya. Gray's type 1 on the other hand is presumably absent from all cell bodies, although it must be recalled that the narrowness of the membrane differentiation of the asymmetrical synapses on these sites is a characteristic of Gray's type 2. The differences are probably due to fixation and staining. On the other hand one could possibly account for these differences in distribution by the greater sampling of cerebral cortex synapses on the large sections used in this study. There is no doubt that symmetrical contacts on spines are rare. Asymmetrical axosomatic contacts are only found on the perikarya of some stellate cells.

The validity of the distinction between Gray's type 1 and type 2 synapses has been sharply criticized by Karlsson because he was unable to make the same distinction in aldehyde fixed lateral geniculate nucleus. Van der Loos's observations in the cerebral cortex led him to the interpretation that Gray's types are but two extremes of a unimodal distribution of synaptic characteristics.

Karlsson's criticism of Gray's types does not really apply since Gray's description is specifically for the cerebral cortex and never implied that the distinction would necessarily hold true in all regions of gray matter throughout the central nervous system.

Van der Loos's criticism is more to the point. The randomly sampled series of 100 synapses reported in the first part of this study shows that the great majority of synapses can be classified as symmetrical or asymmetrical. This suggests a double population at least with respect to the presence or absence of a cytoplasmic postsynaptic opacity contiguous with the postsynaptic membrane.

The extracellular characteristics differentiating Gray's types do not hold true in formalin fixed material. If Van der Loos's criticism applies mainly to the extracellular details observed on his osmium immersed cortex, the formalin fixed tissue would seem to bear him out at least by showing that the extracellular characteristics defining Gray's types are not independent of fixation techniques.

The two types of vesicle population already described by Bodian and by Lund and Westrum in the cerebral cortex have been observed. They are essentially similar to what has been described in many regions of the central nervous system.

Fig. 15. Stellate cell body from the fourth layer of the cat cerebral cortex. Arrows indicate synaptic contacts. × 5,000.
Terminals can easily be classified as containing one or the other type of vesicles in the cerebral cortex in the majority of cases, provided the section includes a sufficient number of vesicles in the terminal and provided that the size as well as the shape of the vesicles is taken into consideration. This is in accord with Larramendi et al.\textsuperscript{15} who has stressed the importance of size in distinguishing between these two types of vesicle populations in the cerebellum. In fact, however, it would seem that the two populations are best characterized by stereoscopic analysis. By this method Gray\textsuperscript{10} has shown that the shape of so-called spheroidal vesicles is truly spheroidal. The structure of flattened vesicles is extremely varied. They are cylindrical, disc-like, doughnut shaped, or tubular. In three dimensions, they are more pleomorphic than flattened.

There is a rather strict association of flattened vesicles with symmetrical contacts and of spheroidal vesicles with asymmetrical contacts in the cerebral cortex, whenever the type of contact and the type of vesicle are both clearly characterized. It is not wished to imply this association will hold true in other regions of the central nervous system.

The terminals containing a large number of granular vesicles probably correspond to Bodian’s\textsuperscript{2} type G terminals.

The distribution of synaptic types described above can be summarized with the help of Fig. 21.

Pyramidal cells (at least those of layer II which have been most extensively studied) receive most of their synaptic contacts on spines. These synapses are mainly of the asymmetrical type with spheroidal vesicles (Fig. 21, closed circles). Some spines do form symmetrical contacts associated with flattened vesicles (Fig. 21, open circles). They are rare, but when found, are too often associated with asymmetrical synapses with spheroidal vesicles on the same spine for this coupling to be fortuitous. The dendritic trunks bearing these spines also receive both types of synaptic complexes. Synapses, however, are relatively few on these dendrites in the sense that vast expanses of dendritic membranes are devoid of contacts.

The cell body, the initial portion of the apical dendrites and the parent stem of basal dendrites (all characteristically devoid of spines) have only been seen to receive symmetrical contacts with flattened vesicles. These synapses are also relatively few in number. On the perikarya only 1–6 at maximum are present per cell body in the plane of section.

At least some stellate cells in layer I and layer IV possess a different synaptology. Asymmetrical synapses with spheroidal vesicles and symmetrical synapses with flattened vesicles are intermingled on many cell bodies and many dendritic trunks

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Fig. 16. Layer I dendritic trunk with many synaptic contacts. × 15,000.
Fig. 17. Layer I dendritic trunk with many synaptic contacts. × 16,500.
Fig. 18. Layer IV dendritic trunk with many synaptic contacts. × 8,000.
Fig. 19. Asymmetrical synapse with spheroidal vesicles on layer I stellate cell body. × 24,000.
Fig. 20. Asymmetrical synapse with spheroidal vesicles on layer IV stellate cell body. × 24,000.
interpreted as belonging to stellate cells. Some of these stellate cells and dendrites receive a 'moderate' number of synapses while others are literally covered by them. More synapses difficult to classify (Fig. 21, half-closed circles) seem to be present on stellate cells than on pyramidal cells.

The foregoing description of the distribution is essentially in accord with Lund and Westrum's\textsuperscript{17} analysis of the olfactory cortex. They have stated that terminals with flattened vesicles contact mainly cell bodies, rarely spines, and that terminals with spheroidal vesicles contact mainly spines, rarely cell bodies.

The overall estimation of the density of synaptic contacts on various cell parts corresponds well with the visualization of bouton distribution as seen by light microscopy\textsuperscript{9}. The precise outlining of a large number of stellate cell bodies and even dendrites in layer IV by many boutons fits well with the large number of contacts found on some of these cells on electron microscopy. Conversely, pyramidal cell bodies and dendrites in layers II and III are not clearly outlined by boutons in optic microscopy. The boutons are diffusely scattered in the neuropil\textsuperscript{5}. This correlates well with the present estimation of a large number of contacts on spines and of relatively few contacts on cell bodies and dendritic trunks.

This distribution enhances the theoretical importance one should attach to the spine and to the spine apparatus. Spines could be thought of as a differentiation which simply increases the surface area of the dendritic membrane to permit a greater number of synaptic contacts. The relative absence of synaptic contacts on spine bearing dendritic

Fig. 21. Schematic drawing illustrating the distribution of synaptic types on stellate and pyramidal cell bodies, dendrites and spines. Closed circles represent asymmetrical contacts with spheroidal vesicles. Open circles represent symmetrical contacts with flattened vesicles. Half-closed circles indicate synapses difficult to classify as one or the other type or exceptions to the described association between types of vesicles and synaptic differentiations.

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trunks shows that this is not the case and strongly suggests that spines may have a much
more specific functional significance. It must be stressed that the pyramidal cells
examined have been mainly from layer II. Large border cells of O'Leary\textsuperscript{19}, giant
pyramidal cells of layer V and some large fusiform cells in the deep layers of the
cortex may have a different synaptology as suggested by light microscopy\textsuperscript{5}. They have
been too rarely encountered in electron microscopy for serious comment.

Similarly it may be that some stellate cell bodies receive only one or the other
type of synaptic endings: this could only be determined by serial sectioning. That the
two types of endings are found on some stellate cell bodies is however obvious without
serial sectioning. The greater number of synaptic contacts on spine free as compared to
spine bearing dendritic trunks has also been seen in the molecular layer of the cere-
bellar cortex\textsuperscript{18}. The only cell bodies present in the molecular layer of the cerebellum
are those of stellate cells. Any dendrite seen in continuity with a cell body is certainly a
stellate cell dendrite. Many of these have been seen to receive a greater density of
synaptic contacts than the spine bearing dendritic trunks of Purkinje cells.

It has cautiously been suggested\textsuperscript{25} that Gray's type 1 may be excitatory and type
2, inhibitory. It has also been suggested that spheroidal vesicles may be related to
excitatory synapses and flattened vesicles to inhibitory synapses\textsuperscript{2,15,34}. There is no
incompatibility between these two theories in the present observations on cat visual
cortex. If they prove to be correct, one would expect a quite different spatial interplay
of excitation and inhibition on pyramidal and stellate cells.

It is hoped that this continuing detailed study of synaptic patterns on different
cell types in the different layers of the cerebral cortex will subsequently permit a
significant analysis of the patterns of degenerated endings after selected lesions of
cortical afferents and thus a better understanding of cortical circuitry.

SUMMARY

Two types of synaptic membrane differentiation can be seen in formalin fixed
cerebral cortex, corresponding closely, but not strictly analogous to Gray's type 1 and
2 described on OsO\textsubscript{4} immersed, PTA stained material. They are referred to as asym-
metrical and symmetrical synapses.

On the whole, asymmetrical synapses are associated with the spheroidal type of
vesicle population, symmetrical synapses with the flattened type of vesicle population.

On pyramidal cells of the second cortical layer, most synaptic contacts are found
on dendritic spines. The vast majority of these spine contacts are of the asymmetrical
type with spheroidal vesicles. Rarely a symmetrical synapse with flattened vesicles is
found coupled to an asymmetrical one on a common spine.

The spine bearing dendritic branches receive comparatively few contacts. Those
present are of one or the other of the afore-said types. The spine-free initial portion of
apical and parent stem dendrites as well as the soma also receive relatively few contacts.
These, however, virtually all appear to be of the symmetrical type with flattened
vesicle.
Stellate cells in the first and fourth layers receive both types of contacts on both their cell bodies and dendrites. Some have relatively few synapses. Others, especially in layer IV are quite remarkably covered by them.

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