FINE STRUCTURE OF THE MEDIAL NUCLEUS OF THE TRAPEZOID BODY OF THE BAT WITH SPECIAL REFERENCE TO TWO TYPES OF SYNAPTIC ENDINGS

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ABSTRACT

The medial nucleus of the trapezoid body has been studied electron microscopically in two species of bat, Miniopterus schreibersi fuliginosus and Vespertilio superans, which were perfused with three different kinds of fixatives, osmium tetroxide, glutaraldehyde, and formaldehyde. Two types of synaptic endings are observed in the nucleus: the abundant calyciferous endings and the less frequently occurring "small-vesicle endings." The former endings vary greatly in size, and contain extended extracellular spaces between pre- and postsynaptic membranes. The latter endings are always small, without the extended extracellular spaces, and tend to lie side by side. In all of the materials perfused with three different fixatives, synaptic vesicles in the calyciferous endings are round in shape and larger than those in the small-vesicle endings. The shape of vesicles in the small-vesicle endings varies according to the kinds of fixatives used; round in osmium tetroxide-fixed materials, flattened in formaldehyde-fixed materials, and somewhat round or flattened in glutaraldehyde-fixed materials. It is suggested that the calyciferous endings are excitatory in nature and that the small-vesicle endings are inhibitory.

INTRODUCTION

In the studies of synapses in the mammalian central nervous system, Uchizono (1965) and Bodian (1966) have proposed an intriguing hypothesis that synaptic vesicles in the excitatory synapses are spherical and that those in the inhibitory synapses are flat shaped. Since then, several investigators have described the existence of two different types of synaptic vesicles in various animals (Atwood and Jones, 1967; Atwood, 1968; Bodian, 1966, 1970; Clark, 1969; Gray, 1969; Halász and Csillik, 1969; Larramendi, Fickenschger, and Lemkey-Johnston, 1967; Lenn and Reese, 1966; Lund and Westrum, 1966; McDonald and Rasmussen, 1969; Nadol and de Lorenzo, 1968; Uchizono, 1966, 1967 a, 1967 b; Walberg, 1966 b). In many cases, however, it has been difficult to correlate function with ultrastructural features. In a few cases such correlation was possible because the function of the synapses was well established by physiological studies: one example pertains to the flat-shaped vesicles contained in the synaptic endings over the dendrites of crustacean stretch receptor neurons, where only inhibitory function has been known (Uchizono, 1967 a; Nadol and de Lorenzo, 1968); another example pertains to the flat vesicles in the inhibitory synapses of the cerebellar cortex (Uchizono, 1967 b; Larramendi, Fickenschger, and

It has been known that the medial nucleus of the trapezoid body, one of the auditory nuclei, receives two kinds of axonal endings, calyceiform endings and small terminal boutons. The former are large endings encircling the perikaryon, the calyces of Held (Held, 1893), and the latter are small axonial endings around the perikaryon and dendrites (Ramón y Cajal, 1909; Moster, 1968). Lenn and Reese (1966), in their electron microscopic study of the cochlear and trapezoid nuclei in the chinchilla and the rat, observed that the shape of the synaptic vesicles of both types of endings is spherical, but that the size of the vesicles in the calyces is larger than that of the vesicles in the small boutons. They suggested that the function of the calyces and the small boutons are excitatory and inhibitory, respectively. This idea, which emphasizes the size of vesicles rather than their shape, seems somewhat different from Uchizono and Bodian's theory.

The present paper will describe the fine structure of the medial nucleus of the trapezoid body of the bat, in which the auditory system is remarkably developed. The morphological characteristics of the two types of synaptic endings in relation to their possible functional roles will be described in detail. The other purpose of this research is to study the effect of different fixatives on the size and shape of the vesicles in the two types of synaptic endings.

MATERIALS AND METHODS

Two species of bats obtained from Tokyo area, Miniopterus schreibersi fuliginosus (nine specimens) and Vespertilio superans (two specimens) were used in this study. The animal was anesthetized with Nembutal, and the brain was fixed by perfusion through the aorta with balanced salt solution followed by either of the following fixatives: 2% osmium tetroxide in 0.2 M phosphate buffer, 4% formaldehyde in 0.2 M phosphate buffer, or 3% glutaraldehyde in 0.2 M phosphate buffer. Each of three Miniopterus bats was perfused with a different fixative. One Vespertilio bat was perfused with glutaraldehyde, and the other with formaldehyde. After the perfusion the desired area of the brain stem was removed, cut into serial transverse slices, and kept in vials containing the same fixative used for the perfusion for an hour in a cold environment. The aldehyde-fixed specimens were then placed into 2% osmium tetroxide in phosphate buffer after being washed in 0.2 M phosphate buffer for approximately 10 min. After dehydration in a graded series of ethyl alcohols, they were embedded in Epon. The medial nucleus of the trapezoid body was identified with the light microscope in transverse thick sections after staining with toluidine blue. The ultrathin sections were stained with lead citrate or with both uranyl acetate and lead citrate, and examined with a Hitachi HU 11B electron microscope.

RESULTS

Microchiropteran bats perceive ultrasonic vibrations and achieve acoustic echo-location (Griffin, 1958), and they, like dolphins, possess extremely large auditory centers compared with other mammals (Poljak, 1926; Ogawa, 1962; Harrison and Irving, 1966). Both species of bats studied here are insectivorous, microchiropteran bats, and the morphologic features of the trapezoid body of both species are very similar.

The medial nucleus of the trapezoid body develops to a large size, extending medially to the raphe (Fig. 1). The nucleus consists of many spherical or oval neurons about 20 μ in diameter (Fig. 2). The neuron has a spherical nucleus, about 10 μ in diameter, in which usually one nucleolus is present (Fig. 2).

The cytoplasm of the neuron contains such organelles as the endoplasmic reticulum, Golgi apparatus, and mitochondria, and these organelles show the typical features of those seen in nerve cells in general. The granular endoplasmic reticulum is distributed throughout the perikaryon, and most of its cisterns are arranged randomly and not in parallel arrays (Figs. 3 and 6). A large number of ribosomes are scattered throughout the cytoplasm (Figs. 3 and 6), and the Golgi apparatus, which is usually observed near the nucleus, is well developed. Multivesicular bodies and dense bodies are frequently present in the perikaryon (Figs. 3 and 8).

Mitochondria are generally numerous. They are usually round or moderately elongated, but sometimes very long, exceeding 10 μ in length. Most of the mitochondria show the usual ultrastructural appearance, but infrequently they contain special profiles, intramitochondrial inclusions (Figs. 3 and 4). These inclusions are located in dilated intracistral spaces, and show an appearance of long, fine dense lines, which are packed together and run parallel with each other. The thickness of the dense lines and the distance between the lines vary greatly. At arrow A of Fig. 4, the dense lines are about 75 A thick and 70-80 A
apart; at arrow B, they are about 90 Å thick and 120 Å apart; and at arrow C, there is no pattern of parallel lines. In possibly cross- and oblique sections (Fig. 3) the inclusion still exhibits a pattern of dense lines, and not any structure that might be considered a cross-section of the filaments could be found. Hence, these inclusions seem to consist of parallel layers of thin lamellae rather than of filaments or fibrils. In nerve cells intramitochondrial inclusions have not been reported, although various types of intramitochondrial inclusions have been found in various kinds of cells: for example, in glial cells (Srebro, 1965; Mugnaini, 1964), hepatic cells (Wills, 1965), and kidney tubule cells (Suzuki and Mostofi, 1967). The functional role of these inclusions is uncertain.

The surface of the nerve cell body is usually surrounded by astrocytic processes and nerve endings. Occasionally, the cell bodies directly contact each other. Such direct contact is found more often in the medial nucleus of *Vespertilio* (Fig. 2) than in that of *Miniopterus*. Electron microscopically, two neurons lie close to each other and are separated by an extracellular space about 200 Å in width. The apposing membranes sometimes form a desmosome-like structure; similar structures have been reported in the cerebellum (Gray, 1961), in the habenula nuclei (Milhau and Pappas, 1966), and in the medullary command nucleus of the skate electric organ (Nakajima, 1970). However, the membranes do not form tight junctions, which are abundant in the central nervous system of electric fish (Bennett, Pappas, Aljure, and Nakajima, 1967).

Extended extracellular spaces filled with moderately electron-opaque material are seen in the medial nucleus, mainly around calyciform endings as described later. Similar extended extracellular spaces have been described in certain other vertebrate brains (Metuzals, 1962; Robertson, Bodenheimer, and Stage, 1963; Bennett, Aljure, Pappas, and Nakajima, 1967; Bennett, Nakajima, and Pappas, 1967; Bennett, Pappas, Giménez, and Nakajima, 1967; Peters, Proskauer, and Kaiserman-Abramof, 1968; Gray, 1969). In the present study the material contained in the extended extracellular spaces is less dense than the basement membrane around the capillaries (Fig. 5), and the spaces are separated from the basement membrane by astrocytic processes (arrow, Fig. 5).

**Nerve Endings**

Most of the synapses in the nucleus are located on the nerve cell body. The size of presynaptic nerve endings is variable. Typical calyx-like endings of Held, demonstrated in silver-impregnated, light microscopic sections (Held, 1893; Ramón y Cajal, 1909), are only occasionally
The scale line in all electron micrographs represents 1 μ except in Fig. 4.

**Figure 3** Electron micrograph of a part of a perikaryon. *Miniopterus schreibersi fuliginosus* fixed by perfusion with osmium tetroxide. Note the intramitochondrial inclusions (IMI), which show lamellar subunits running parallel to each other. M, mitochondria; DB, dense bodies; SVE, small-vesicle ending; GER, granular endoplasmic reticulum; NF, neurofilaments. X 6,100.

**Figure 4** Electron micrograph of intramitochondrial inclusions. *Miniopterus schreibersi fuliginosus* fixed by perfusion with osmium tetroxide. The thickness of the lamellar elements which constitute the inclusion and the distance between the neighbouring lamellae vary from place to place. At arrow A the inclusion consists of lamellae about 75 Å thick and 70–80 Å apart; at arrow B it consists of lamellae about 90 Å thick and about 120 Å apart; and at arrow C there is no clear lamellar structure. The scale line represents 0.1 μ. X 100,000.
FIGURE 5 Electron micrograph showing extended extracellular spaces (EES) filled with amorphous electron-opaque material around calyciferous endings. Miniopterus schreibersi fuliginosus fixed with osmium tetroxide. An extended extracellular space is separated from the basement membrane (BS) of the capillary by an astrocytic process (arrow), and its density is lower than that in the basement membrane. CE, calyciferous ending; P, perikaryon; M, mitochondria. × 88,600.

observed. This is due to the difference in the thickness between light and electron microscopic sections. The electron microscopic sections are so thin that only very favorable planes of sections could reveal the whole picture of complex structures like calyx endings. Figs. 6 and 8 show typical calyx-like endings, which make multiple synaptic contacts with the perikaryon. They contain the usual organelles found in the chemical synapses, i.e., abundant mitochondria and numerous synaptic vesicles. The vesicles are generally round in shape and rather large, the average diameter being 480–460 Å (Table I). One characteristic of this type of synaptic ending is, besides its large size and calyx-like shape, the presence of the extracellular spaces, which are interposed at several places between the pre- and postsynaptic membranes (Figs. 5–8). The space is filled with a moderately electron-opaque material. Although the extended spaces around the endings are found regardless of the kinds of fixatives used, the density of the material in the spaces was the highest with osmium tetroxide fixation (Figs. 5–7) and the lowest with formaldehyde (Fig. 8).

Fig. 5 (CE) shows a medium-sized ending. It has almost the same characteristics that have been described for a typical, large calyx-like ending: i.e., the presence of extended extracellular spaces and large, round-shaped synaptic vesicles. The size distribution of this type of ending, the common feature of which is the presence of large, round vesicles and the extended extracellular space, is quite extensive, and a whole range of size from a typical calyx-like ending to a small ending, such as shown in Fig. 7 (CE), was observed. Hence, it seems reasonable to assume that these endings form one system of nerve endings, calyx-like endings of Held (1893), which Harrison and Irving (1964) demonstrated to be afferent terminals of the cochlear nuclei. Many medium-to-small-sized nerve endings perhaps represent profiles of the calyx-like endings on skewed sections, or just a part of the calyx. The other possibility would be the collaterals from the calyx-
like endings or from the afferent axons. In fact, with the rapid Golgi method, Morest (1968) demonstrated the presence of such collaterals in the medial nucleus of the trapezoid body of the cat. In other words, the size distribution of this type of ending merely represents a complex structure of the calyx-like endings, and thus, these endings, regardless of their size, will be referred to as "calyciferous endings" (CE).

There is another type of nerve ending on the nerve cell, as described by Lenn and Reese (1966) in the cochlear and trapezoid nuclei of the rat. This type will be called "the small-vesicle ending" (marked SVE in Figs. 6, 7, and 9-12). The small-vesicle endings (SVE) are encountered less frequently than the calyciferous endings. They are always small in size and have none of the characteristics of the calyciferous endings. Thus, the extended extracellular spaces are never present between the pre- and postsynaptic membranes; these membranes are always separated rather evenly by a gap of only about 200 Å. The synaptic vesicles are about 390-340 Å in size and are smaller than those in the calyciferous endings. This is clearly seen in the osmium tetroxide-fixed materials (compare SVE and CE in Fig. 6), in which the vesicles of both types of endings are round. In the formaldehyde-fixed materials, the vesicles of SVE become flattened in shape (Figs. 9 and 10) and are more readily distinguishable from those in the calyciferous endings. The differences in the shape and size of the vesicles in the different fixatives will be dealt with in more detail.

There are additional differences between both types of endings. The SVE tend to lie side by side and are separated by a gap of only about 200 Å (Figs. 6, 10, and 11), as described by Lenn and Reese (1966). On the other hand, the calyciferous endings, when lying near each other, are separated by extended extracellular spaces (Fig. 5) or glial cell processes. The two types of endings also differ with respect to the number of neurofilaments. Many more neurofilaments are usually encountered in the calyciferous ending than in the SVE, as described by Lenn and Reese (1966). For example, in Fig. 7 the calyciferous ending

### Table I

Shape and Size of Synaptic Vesicles in the Medial Nucleus of the Trapezoid Body

Each figure is obtained from measurements on 200 vesicles.

<table>
<thead>
<tr>
<th>Ending</th>
<th>Fixation</th>
<th>Mean of maximum diameter (M)</th>
<th>Mean of minimum diameter (m)</th>
<th>Mean of vesicle elongation index (M/m) ± SEM</th>
<th>Mean of diameter (A) ± SEM</th>
<th>Mean of vesicle area (xMm) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calyciferous endings</td>
<td>OsO₄</td>
<td>520</td>
<td>460</td>
<td>1.2 ± 0.01</td>
<td>480 ± 1</td>
<td>18 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Glutaraldehyde</td>
<td>510</td>
<td>440</td>
<td>1.2 ± 0.01</td>
<td>470 ± 2</td>
<td>18 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Formaldehyde</td>
<td>500</td>
<td>430</td>
<td>1.2 ± 0.01</td>
<td>460 ± 2</td>
<td>17 ± 0.2</td>
</tr>
<tr>
<td>Small-vesicle endings</td>
<td>OsO₄</td>
<td>430</td>
<td>360</td>
<td>1.2 ± 0.02</td>
<td>390 ± 4</td>
<td>13 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Glutaraldehyde</td>
<td>440</td>
<td>320</td>
<td>1.4 ± 0.02</td>
<td>370 ± 3</td>
<td>11 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Formaldehyde</td>
<td>440</td>
<td>270</td>
<td>1.7 ± 0.03</td>
<td>340 ± 2</td>
<td>9.4 ± 0.2</td>
</tr>
</tbody>
</table>

**Figure 6**  Electron micrograph of two large calyciferous endings (CE) and two small-vesicle endings (SVE). *Miniopterus schreibersi fuliginosus* fixed by perfusion with osmium tetroxide. The calyciferous endings with synaptic contact on perikaryons (P) contain round synaptic vesicles, coated vesicles (CV), and mitochondria (M). Notice the extended extracellular spaces (EES) between the calyciferous endings and the perikaryons. The two small-vesicle endings are on a perikaryon and a dendrite (D), lie side by side, and contain round synaptic vesicles smaller than those in calyciferous endings, coated vesicles (COV), and mitochondria (M). No extended extracellular spaces are observed between these small-vesicle endings and the perikaryon. × 38,500.
shows many more cross-sections of neurofilaments (NF) than the small-vesicle ending.

Almost all the synaptic vesicles are smooth-surfaced without dense cores. However, occasionally coated vesicles, their diameters ranging between 200 and 800 Å, are encountered in both types of nerve endings (Fig. 6). Coated invaginations of the presynaptic membranes are also rarely encountered. Coated vesicles and invaginations have been reported on both the presynaptic (Andres, 1964) and postsynaptic sides of the synaptic membranes of the central nervous system (Waxman and Pappas, 1969; Nakajima, 1970), and it has been suggested that they have a functional role in the uptake of extracellular substances such as protein (Andres, 1964; Waxman and Pappas, 1969).

Cored vesicles (COV) with diameters of about 1000 Å occur occasionally in the small-vesicle endings (Figs. 6 and 10, COV).

Size and Shape of Synaptic Vesicles

It has been suggested that the flat-shaped vesicles occur with aldehyde fixation (Larramendi, Fickenscher, and Lemkey-Johnston, 1967; Walberg, 1966 b), particularly with formaldehyde fixation (Lund and Westrum, 1966). Consequently, the author has investigated quantitatively the effects of different fixatives, osmium tetroxide, glutaraldehyde, and formaldehyde, on the shape and size of synaptic vesicles of both types of endings. Table I and Fig. 13 summarize the results.

Measurements on 200 vesicles in each type of synapse were made for each differently fixed bat (Miniopterus schreibersi fuliginosus). In several micrographs of each type of ending, areas were arbitrarily chosen and measurements were made on all the vesicles contained in each area until the number in each case reached 200. Only agranular, smooth-surfaced vesicles were included in the measurements. When readily distinguished, the tubular endoplasmic reticulum was not measured, but it is possible that its transverse sections might have been included in the measurements. The shape of the vesicles is represented by the vesicle elongation index, the ratio of the maximum diameter (M) to the minimum diameter (m) for a given vesicle. Thus, a larger index means a flatter vesicle. The diameter is the geometrical mean of M and m, that is, \( \sqrt{Mm} \). The area of the vesicles was calculated by assuming an elliptical shape by the formula \( \pi Mm/4 \).

As shown in Table I, the shape of synaptic
FIGURE 8  Electron micrograph of a large calyciferous ending (CE) with multiple synaptic contacts on a perikaryon (P). Miniopterus schreibersi fuliginosus fixed by perfusion with formaldehyde. The ending contains round synaptic vesicles, neurotubules (NT), neurofilaments (NF), and mitochondria (M). Extended extracellular spaces (EES) are present between the ending and the perikaryon. In the perikaryon, granular endoplasmic reticulum (GER), a multivesicular body (MV), neurotubules (NT), dense bodies (DB), and mitochondria (M) are seen. × 8,000.

FIGURE 9  Electron micrograph of a small-vesicle ending (SVE) and a part of a calyciferous ending (CE) with synaptic contact on a perikaryon (P). Miniopterus schreibersi fuliginosus fixed by perfusion with formaldehyde. The calyciferous ending contains round and large synaptic vesicles, and the small-vesicle ending contains elongated, smaller synaptic vesicles. No extended extracellular spaces are observed between the small-vesicle ending and the perikaryon. M, mitochondria. × 37,400.

FIGURE 10  Electron micrograph of three small-vesicle endings (SVE) with synaptic contact on a perikaryon (P). Miniopterus schreibersi fuliginosus fixed by perfusion with formaldehyde. The endings contain small elongated synaptic vesicles, mitochondria, and cored vesicles (COV). They lie side by side, separated by a gap about 200 Å in width. × 40,400.
vesicles in the calyciferous endings is rather spherical, and the elongation index does not change according to the different fixatives (the mean elongation index was always 1.2). However, the shape of vesicles in the SVE is, to a large extent, dependent on the fixatives: in the osmium tetroxide-treated materials the shape is round (the index = 1.2), but in the formaldehyde-treated materials it is markedly elongated (the index = 1.7). The difference is highly significant. In most of the glutaraldehyde-fixed preparations the shape is somewhat elongated (the index = 1.4). However, fixation with glutaraldehyde produced variable results, and in one bat the vesicles were elongated as much as in the case of formaldehyde fixation (Fig. 12). The shapes of the vesicles in any particular ending were mixed, and this variability was greater in the SVE, as

Figure 11 Electron micrograph of a calyciferous ending (CE) and two small-vesicle endings (SVE) with synaptic contact with a perikaryon (P). Miniopterus schreibersi fuliginosus fixed with glutaraldehyde. The calyciferous ending contains round and large synaptic vesicles. The small-vesicle endings contain round and smaller vesicles, and they lie side by side. Note the presence of an extended extracellular space (EES) between the calyciferous ending and the perikaryon, and the absence of such spaces between the small-vesicle endings and the perikaryon. DB, dense bodies. × 35,000.

Figure 12 Electron micrograph of a small-vesicle ending (SVE) and a calyciferous ending (CE) with synaptic contact on a perikaryon (P). Miniopterus schreibersi fuliginosus fixed by perfusion with glutaraldehyde. The calyciferous ending contains large, round vesicles, while the small-vesicle ending contains elongated, smaller vesicles. × 39,700.
indicated by the greater standard errors of the mean (SEM) for the vesicle elongation index. At least part of this variability can be ascribed to the three-dimensional figures of the vesicles: assuming that a vesicle has the figure of an ellipsoid, its plane sections would be ellipses or circles according to the direction of the section.

From Fig. 13 and Table I, it is also evident that the mean diameter and the mean area of vesicles is significantly ($P < 0.01$) larger in the calyciferous endings than in the SVE, and this is true in all three differently fixed materials.

In the calyciferous endings the diameter and area of the vesicle do not differ much according to the fixative used (area $= 17.0 \sim 18.0 \times 10^4 \mu^2$). On the other hand, the diameter and area of the vesicles in the SVE appear to be smaller with glutaraldehyde or formaldehyde fixation than with osmium tetroxide (Table I, Fig. 13). At first sight, this result seems to indicate that the volume of the vesicle in the SVE becomes smaller in formaldehyde or glutaraldehyde than in osmium tetroxide. However, this conclusion should be interpreted carefully, since there is a possibility that the mean vesicle area becomes smaller when it is flat-shaped than when it is round, even though the volume under both conditions is the same. Nevertheless, it is obviously reasonable to conclude that the volume of the vesicles in the calyciferous endings is significantly larger than that in the SVE, regardless of the variable shape of the vesicles in the SVE.

DISCUSSION

Nerve Endings

The present study has demonstrated that there are two types of synaptic endings, the calyciferous and the small-vesicle endings (SVE), in the medial nucleus of the trapezoid body of the bat. Using the silver staining method and the degeneration study, Stotler (1953) and Harrison and Irving (1964) concluded that the calyces of Held in the nucleus of the trapezoid body are afferent fibers from the contralateral cochlear nucleus. By the electrophysiological method, Galambos, Schwartzkopff, and Rupert (1959) found that the majority of the action potentials recorded from the trapezoid nucleus are activated by contralateral sound stimulation. Therefore, it seems very likely that the calyciferous endings described here correspond to the excitatory synapses whose input comes directly from the cochlear nucleus.

On the other hand, the origin of the small-vesicle endings is not yet known anatomically. However, in their recent electrophysiological study on the nucleus of the trapezoid body of the cat, Watanabe, Liao, and Katsuki (1968) reported the existence of a long-lasting inhibitory response, probably mediated by interneurons which fire repetitively. Since the calyciferous endings are considered to come from the cochlear nucleus and are excitatory, the SVE might be correlated with this inhibitory function mediated by the interneurons. Another source of this type of bouton would be the higher auditory centers, which might exert an inhibitory influence, as in the case...
of the cochlear nucleus (Rasmussen, 1960; Pfalz, 1962). However, the existence of such descending fibers has so far not been demonstrated in this nucleus. Similar small endings containing small and/or flattened vesicles have also been reported in other auditory nuclei: the ventral cochlear nucleus (Lenn and Reese, 1966; McDonald and Rasmussen, 1969) and the medial superior olive (Clark, 1969). In both nuclei the function of these small endings has been suggested to be inhibitory.

Size and Shape of Synaptic Vesicles

The present study has revealed that the vesicles in the calyciferous endings are always round in shape. On the other hand, the small vesicles in the SVE (possibly inhibitory) become flattened only in formaldehyde and glutaraldehyde. Thus, there seem to be differences in the content of vesicles with respect to susceptibility to chemicals between the two types of vesicles. This conclusion is in agreement with that of Lund and Westrum (1966), Larramendi, Fickenscher, and Lenkey-Johnston (1967), and Bodian (1970) who observed a similar phenomenon in synapses in the olfactory cortex, the superior colliculus, the cerebellum, and the spinal cord. Bodian (1970) further classified the types of vesicles into three major types according to their reaction to fixatives and buffer washing. It is important to note that this flattening of vesicles is different from the flattening of vesicles reported by Walberg (1966 a) in the degenerating synapses, because the latter type of flattening occurs in all synapses including the ones containing large, round vesicles in normal condition.

In spite of the variability of the shape of the SVE vesicles according to the fixatives, their size was always smaller than that of the vesicles of calyciferous endings. It is difficult to speculate which fixatives retain the true shape of vesicle in the living animal. Therefore, at this stage it seems more reasonable to state that size rather than shape is the basic structural difference between the two types of synaptic vesicles. However, from the practical point of view, the shape of synaptic vesicles in formaldehyde-fixed materials seems to be a convenient means of differentiating the two types of vesicles, because flattening is the remarkable and specific reaction of the smaller vesicles; on the other hand, the difference in the size of the vesicles is not very apparent unless one plots the size distribution for a large number of vesicles.

In this paper, the two types of synaptic endings which are distinguished from each other on the basis of presynaptic structure have been discussed in relation with postsynaptic physiological responses, excitation, and inhibition. Strictly speaking, however, it is more reasonable to correlate the presynaptic structures with the identification of the transmitter substances released from the endings, since presynaptic physiological features are not always parallel to the postsynaptic ones. For example, acetylcholine, a kind of transmitter, produces either excitatory or inhibitory responses in different subsynaptic structures: excitatory responses in the neuromuscular junction (del Castillo and Katz, 1955) and inhibitory ones in the cochlea and lateral line organs (Fex, 1968; Russel, 1968) or the heart (Hutter and Trautwein, 1956). However, we are almost ignorant about the real identity of transmitter substances in many synapses of the central nervous system, and usually only the postsynaptic responses are known. Furthermore, with the exception of acetylcholine and some catecholamines (McLennan, 1970; Salmoiraghi, 1966), most of the transmitter substances, such as glutamate and gamma aminobutyric acid, are known to always produce one kind of postsynaptic response, either excitation or inhibition. Hence, it seems permissible, and at the present time very reasonable, to correlate the morphology of the presynaptic terminals with the functional manifestation of the postsynaptic membrane.

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