Bipolar neurons in rat visual cortex: a combined Golgi–electron microscope study

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Summary

Golgi-impregnated bipolar neurons in rat visual cortex have been examined by both light and electron microscopy. Bipolar neurons are encountered throughout layers II to V and are recognized by their spindle-shaped cell bodies and vertically elongate, narrow dendritic trees which may traverse the cortex from layer II to layer V. Although a single primary dendrite usually extends from each end of the cell body, two primary dendrites may extend from one pole, usually the lower one, and an additional short dendrite may emerge from one side. In the electron microscope gold-toned Golgi-impregnated neurons are seen to have folded nuclear envelopes and except at the poles of the cell body where the dendrites emerge, the nucleus is surrounded by only a thin rim of cytoplasm. Both the cell body and the dendrites form asymmetric and symmetric synapses. Usually the axon of a bipolar neuron arises from one of the primary dendrites and it soon assumes a vertical orientation, to either descend or ascend through the cortical neuropil. Some bipolar neurons have myelinated axons and only the initial portion is impregnated in Golgi preparations, but when they are unmyelinated the axons can be seen to form vertical plexuses and asymmetric synapses. Most commonly the terminals synapse with dendritic spines, some of which are derived from apical dendrites of pyramidal cells, but other terminals synapse with the shafts of apical dendrites, and with the cell bodies and dendrites of nonpyramidal cells.

It is apparent that these bipolar neurons are the cells which others have shown to label specifically with antisera to vasoactive intestinal polypeptide (VIP), and it is suggested that the prime role of these cells in the cerebral cortex is to excite the clusters of pyramidal cells.

Introduction

The visual cortices of mammals have been the focus of many studies, some concerned with their efferent and afferent connections, and others with the responses of neurons to various visual stimuli and the disposition of various neurotransmitters within neurons and their axon terminals. Yet, we possess only fragmentary knowledge about the synaptic relations of the different morphological types of neurons present within the visual cortex, so that it is still not possible to understand and interpret much of the data...
derived from physiological and other experimental studies in terms of the connections between neurons.

The focus of our studies on connections between neurons has been area 17 of rat visual cortex. This cortex may lack the functional columns of neurons present in visual cortices such as those of the cat (for example, Hubel & Wiesel, 1963) and monkey (for example, Hubel & Wiesel, 1977), but like these cortices it contains neurons with simple and complex visual receptive fields (Madar, 1980; Parnavelas et al., 1980). On the other hand, it seems to contain fewer morphological varieties of neurons than the visual cortices of the cat (for example, Cajal, 1922; O'Leary, 1941; Lund et al., 1979) and monkey (for example, Lund, 1973). Consequently from the point of view of examining the morphological interconnections between neurons, rat visual cortex provides a relatively simpler model. In addition, it has the advantage of being smooth and only about 1.5 mm thick.

On the basis of light microscopic examination of material impregnated by the Golgi technique the neurons of area 17 of rat visual cortex can be described as either pyramidal or non-pyramidal in form (Parnavelas et al., 1977; Feldman & Peters, 1978; Werner et al., 1979), and in an electron microscope study Winfield et al. (1980) have been able to show that about 30% of neurons in rat visual cortex are nonpyramidal. However, there are different types of nonpyramidal cells and they can be classified according to two features, their dendritic patterns, and the abundance of dendritic spines (Feldman & Peters, 1978). Using this classification as a basis we have begun a comprehensive study of neurons in rat visual cortex employing a technique which allows Golgi-impregnated neurons to be examined with both the light and electron microscopes (Fairén et al., 1977). So far we have examined the smooth and sparsely spinous multipolar and bitufted neurons with unmyelinated axons (Peters & Fairén, 1978; Peters & Proskauer, 1980a) and myelinated axons (Peters & Proskauer, 1980b), and have shown that the axon terminals of the neurons with unmyelinated axons form symmetric synapses with a variety of postsynaptic elements. This disposition of the axon terminals is in contrast to that of the chandelier cells studied in this same cortex by Somogyi (1977), for although the axons of chandelier cells also form symmetric synapses, they synapse with only the axon initial segments of pyramidal neurons. Despite these differences in the terminal distribution, however, axon terminals of both the smooth and sparsely spinous multipolar neurons and the chandelier cells label with an antibody to glutamic acid decarboxylase (Ribak, 1978; Peters & Proskauer, unpublished data). Glutamic acid decarboxylase is the enzyme which synthesises γ-aminobutyric acid (GABA) which is considered to be an inhibitory neurotransmitter in mammalian neocortex.

The purpose of the present report is to examine the morphology and synaptic connections of another type of nonpyramidal neuron in rat visual cortex, namely the bipolar cell. Recent studies by Emson & Lindvall (1979), Loren et al. (1979), Sims et al. (1980), and Fahrenkrug (1980) show that bipolar neurons give a positive reaction when pieces of cortex are incubated with antisera to vasoactive intestinal polypeptide (VIP).
Materials and methods

Animals used in this study were Sprague-Dawley albino rats between 20 days and six months of age. The rats were anaesthetized and perfused through the ascending aorta with solutions of paraformaldehyde and glutaraldehyde in 0.08 M cacodylate buffer at pH 7.2, containing 0.03 M calcium chloride (Fairen et al., 1977). The perfused animals were stored overnight in the refrigerator and next day pieces of occipital cortex containing the primary visual cortex, area 17 (Krieg, 1946; Schober & Winkelmann, 1975; Feldman & Peters, 1978), were removed for impregnation by the rapid Golgi technique, using the solutions recommended by Valverde (1970). After impregnation the pieces of visual cortex were transferred through a graded series to pure glycerol, embedded in 7% agar and 100–150 μm thick sections cut using a Sorvall tissue chopper. Sections were taken in the frontal plane, perpendicular to the pial surface, and suitably impregnated bipolar neurons were gold toned and deimpregnated (Fairen et al., 1977). After postfixation in 2% osmium tetroxide the sections were stained en bloc with alcoholic uranyl acetate, dehydrated and embedded in flat sheets of Epon–Araldite.

In three additional two-month-old rats, stereotactic lesions were placed in the left lateral geniculate nucleus and the animals allowed to survive for three days before being perfused and their visual cortices treated according to the protocol given in the preceding paragraph. These rats were used to determine whether geniculocortical axons synapse with bipolar neurons (see Peters et al., 1977, 1979).

After being flat-embedded, gold-toned bipolar neurons were examined and drawn, using a Zeiss light microscope equipped with a drawing tube. The neurons were then remounted on plastic blanks, serially thin sectioned and examined with the electron microscope (see Fairen et al., 1977; Peters & Fairen, 1978).

In series of thin sections, profiles of portions of the gold-toned and deimpregnated neurons were identified by the presence of gold particles within their cytoplasm. On the basis of this study it was found possible to identify some unimpregnated bipolar cells within the series of thin sections, and a number of electron micrographs of these unimpregnated neurons were taken to provide additional information about their morphology.

Description

Cell bodies and dendrites

In Nissl preparations of area 17 of rat visual cortex the distribution of the cell bodies of neurons into laminae is readily apparent, and except for the one between layers II and III, the boundaries between the cortical layers are quite distinct (Schober & Winkelmann, 1975; Peters & Feldman, 1978). Correlations between the Nissl and Golgi images show that the cell bodies of bipolar neurons are located throughout layers II to V, although bipolar neurons with cell bodies in layer IV were most frequently impregnated.

An appreciation of the forms of the bipolar neurons can be obtained by examination of Fig. 1. This shows camera lucida drawings of eight bipolar neurons which have been examined first with the light microscope and then with the electron microscope. The cell bodies of these neurons are fusiform or spindle-shaped, with their long axes oriented in a vertical direction with respect to the pial surface, and as found previously (Feldman & Peters, 1978), the cell bodies are of relatively uniform size. Indeed the dimensions of the cell bodies vary only between 9 and 12 μm for the minor axis and 18–25 μm for the
Fig. 1. Camera lucida drawings of eight Golgi-impregnated bipolar cells which were examined by both light and electron microscopy. The boundaries between the layers are indicated by the horizontal lines and the numbers of the layers are given in Roman numerals on the left.
 Cortical bipolar neurons

major axis. In general, each end of the elongate cell body tapers into a single primary dendrite, which follows an essentially vertical trajectory, sometimes remaining unbranched, or bifurcating only once to produce a very elongate and narrow dendritic field (for example, cells a, g and h). In other examples, two primary dendrites emerge from one pole and the ascending dendrites produce a narrow dendritic tree while the descending ones branch more profusely and splay out laterally (cells c-f). Occasionally an additional dendrite may emerge from the side of the cell body to extend laterally (for example, cell g), but this dendrite is usually shorter than the others and does little to affect the predominantly vertical and narrow dendritic distribution pattern.

Although bipolar neurons consistently have few primary dendrites, there is great variability in how far these dendrites extend vertically. Thus some of the longer cells, such as cells g and h, stretch from upper layer II to lower layer V, an overall distance of about 1 mm, which is about two-thirds of the depth of the visual cortex. In contrast neurons such as cell a, which has its cell body in the upper part of layer II/III, extend for only about 200 μm, reaching from layer I into layer IV, but the length of most bipolar neurons is between these two extremes. As a group, the bipolar neurons are the slimmest and most vertically extensive of the nonpyramidal cells, their vertical extent only being matched by the pyramidal neurons with their long apical dendrites.

Although many of the bipolar neurons have smooth-surfaced dendrites (for example cells d and f), it is not uncommon for at least a few thin spines to be present (for example, cells a and h), and in our earlier survey (Feldman & Peters, 1978), it was concluded that cells with smooth versus sparsely spinous dendrites occur in about equal numbers.

In thin sections profiles of the various parts of the gold-toned bipolar neurons are marked by their content of gold particles, which are preferentially located beneath the plasma membrane. Sections through the cell bodies of bipolar neurons show the nuclei to be elongate in the direction of the long axis of the somata (Figs. 2, 3) and to contain a single large nucleolus. The nuclei all have rather irregular outlines and the nuclear envelope invaginates to form a cleft running parallel to the length of the nucleus (Fig. 2). The cleft may not be seen in every thin section, but if a series of thin sections through the cell body is examined the cleft becomes apparent. This irregularity and folding of the nuclear envelope is also displayed by other types of nonpyramidal neurons in rat visual cortex (see Peters & Fairén, 1978). It is a feature which often helps to make a preliminary distinction between nonpyramidal and pyramidal neurons, for pyramidal cells nearly always have spherical nuclei and rarely display more than slight irregularities of the nuclear envelope.

Commonly the perikaryal cytoplasm of the bipolar cells is not very electron dense and except at the upper and lower poles of the cell body it is confined to a thin rim surrounding the nucleus. Usually the rough endoplasmic reticulum is not well developed and appears as a few cisternae, some of which may be very long and lie parallel to the surface of the nuclear envelope. At the poles of the cell body, however, where the cytoplasm is more abundant, the cisternae of rough endoplasmic reticulum
may be organized to form stacks in which two or three cisternae lie parallel to each other (Fig. 2). With the exception of cell b (Fig. 1) this is the arrangement of the rough endoplasmic reticulum in all of the gold-toned bipolar cells examined with the electron microscope. In cell b the cytoplasm at the poles of the cell body contains very well organized and extensive cisternae of rough endoplasmic reticulum with many free ribosomes between the cisternae (Fig. 3).

The bipolar cell perikaryon also contains a few mitochondria and lysosomes, but the Golgi apparatus is not usually very obvious, and is mainly confined to small groups of cisternae and vesicles in the cytoplasm at the bases of the dendrites (Fig. 2, G). The cytoplasm at the poles of the cell body also contains fascicles of microtubules passing towards the dendrites (Fig. 2, m). In the perikaryal cytoplasm the microtubules intermingle with a few cisternae of rough endoplasmic reticulum and other perikaryal organelles. However, within a short distance from the bases of the dendrites there is a decrease in the number of cisternae and ribosomes, so that the dendritic cytoplasm soon becomes dominated by microtubules and occasional mitochondria (Figs. 6 and 7, D). Small groups of ribosomes and small cisternae of rough endoplasmic reticulum may still be present in the cytoplasm of the proximal portions of the dendrites, especially at the periphery where there are slight bulges of the dendritic plasma membrane, but as the dendrites become thinner such organelles become much less common. Frequently the vertically oriented dendrites of the bipolar neurons lie parallel to, and intermingled with, the clusters formed by the apical dendrites of pyramidal cells (for example, Peters & Walsh, 1972; Feldman & Peters, 1974; Winkelmann et al., 1975), and in their cytoplasmic features the dendrites of these two types of neurons are very similar; both contain fewer microtubules than the dendrites of the multipolar and bitufted non-pyramidal cells with smooth or sparsely spinous dendrites (Peters & Fairén, 1978; Peters & Proskauer, 1980a).

As seen with the light microscope (Fig. 1), dendrites of some bipolar cells may have a few spines, and when observed in thin sections these spines are quite slender and only rarely end in a bulbous enlargement.

Both symmetric and asymmetric synapses (Colonnier, 1968) occur on the cell bodies of the bipolar cells (Figs. 2, 3). The asymmetric synapses are formed by rather small axon terminals (Fig. 4, At) and the synaptic junctions, which occupy most of the interface between the axon terminal and the perikaryal plasma membrane, have a prominent postsynaptic density (Fig. 4, arrow). Sometimes the asymmetric synapses involve short somatic protrusions, or spines as shown in Fig. 3 (arrow) on the left side of cell b, although not all somatic spines appear to form synapses. The symmetric synapses, on the other hand, are usually formed by larger axon terminals which contain more

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Fig. 2. Electron micrograph of the cell body of cell f. The nucleus (N) has irregular contours. Within the perikaryon profiles of the Golgi apparatus (G), rough endoplasmic reticulum (ER), and microtubules (m) are indicated. The cell body forms a number of asymmetrical (arrows) and symmetrical (arrowheads) synapses. × 10 000.
dispersed and smaller synaptic vesicles (Fig. 4, At). Another difference is that the synaptic junctions of the symmetric synapses (arrowheads) are shorter and less prominent than those of the asymmetric synapses. They occupy only a portion of the interface between the axon terminal and the perikaryal plasma membrane, which may also show the presence of puncta adhaerentia (Peters et al., 1976).

A feature of these bipolar neurons is the rather low incidence of axosomatic synapses. Thus, when thin sections passing through the middle of the cell bodies of the eight gold-toned neurons are examined, only three–five profiles of synapsing axon terminals are usually encountered (Fig. 3). However, there may be as many as seven (Fig. 2) or as few as two synapsing axon terminals visible in any single thin section taken through the soma, and in a sample of 100 axosomatic synapses the ratio of symmetric to asymmetric synapses is three to one.

There is also a similar low incidence of synapses along the shafts of the dendrites of bipolar cells (Figs. 6, 7). The tendency is for symmetrical synapses to occur most frequently on the proximal portions of the dendrites (Fig. 6) and for a higher proportion of asymmetrical synapses to be present along the distal portions of dendrites (Fig. 7), and indeed, along the most distal lengths of the dendrites symmetric synapses are infrequent.

One potential source of axon terminals forming asymmetric synapses with portions of bipolar cells contained in lower layer III and layer IV of area 17 is the lateral geniculate nucleus (Peters et al., 1977). Such synapses can be identified by making a lesion in the lateral geniculate nucleus. A degenerating axon terminal synapsing with the shaft of a gold labelled dendrite from a bipolar neuron is shown in Fig. 5. The cell body of this particular bipolar cell is in upper layer IV and the portion of the dendrite shown in Fig. 5 is from the ascending dendrite at the level of the border between layer II/III and layer IV. A second degenerating geniculocortical axon terminal also synapsed with a descending dendrite of this neuron within lower layer IV.

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Fig. 3. Electron micrograph of the cell body of cell b. The nucleus (N) contains a prominent nucleolus, and in this particular neuron the rough endoplasmic reticulum (ER) is readily apparent at the poles of the perikaryon. Symmetrical axo–somatic synapses are indicated by arrowheads. A somatic spine on the left side of the cell body forms an asymmetrical synapse (arrow). × 9500.

Fig. 4. Electron micrograph showing a portion of the cell body of cell f. One axon terminal (At1) is forming an asymmetric synapse (arrow), while two other axon terminals (At) are forming symmetric synapses (arrowheads). × 40 000.

Fig. 5. Electron micrograph of a gold-toned dendrite (D) from a bipolar cell synapsing with a degenerating geniculocortical axon terminal (At). The terminal is surrounded by an astrocytic process (As). × 40 000.

Fig. 6. Electron micrograph of the proximal portion of the ascending dendrite (D) of cell f. Two axon terminals (At) form synapses with the dendrite. × 30 000.

Fig. 7. Electron micrograph of a distal portion of the descending dendrite of cell g. Although the junctions are somewhat masked by the heavy gold deposit, four axon terminals (At) are forming asymmetric synapses with the dendrite. × 25 000.
Axons
As stated in the previous account (Feldman & Peters, 1978), the axons of bipolar neurons often originate from a primary dendrite, usually a descending one (Fig. 1). Only infrequently do axons emerge directly from the cell body, and an example of this is provided by cell b (Fig. 1). The ascending dendrite of this neuron was cut off close to the cell body, so that the ascending process shown in the drawing is the axon. The axons of other bipolar neurons, such as cells f and h, emerge from a primary dendrite close to the cell body, but it is not unusual, as with cells a, c and g, for the axon to originate as far as 30 \( \mu m \) away from the cell body.

In cell f, only a 30 \( \mu m \) length of axon impregnated and in the electron microscope it became apparent that the impregnation stops where the axon enters a myelin sheath. Another example of a bipolar neuron with a myelinated axon is given in Figs. 8–10. This neuron is not impregnated, but its bipolar form is evident in serial thin sections, which show vertically oriented dendrites emerging from the upper and lower poles of the elongate cell body (see Fig. 8). The axon (Ax) of this neuron originates from the ascending dendrite (D) about 20 \( \mu m \) from the cell body (Fig. 8). At higher magnification (Fig. 9), it is evident that close to the site of origin of the axon (Ax) there is some fasciculation of microtubules (m) in the cytoplasm of the dendrite (D), and these fascicles pass into the axon initial segment, which is also characterized by an undercoating beneath the axolemma (for example, Palay et al., 1968; Peters et al., 1968). The axon initial segment then curves to assume a vertical trajectory and it ascends for a distance of about 30 \( \mu m \) before entering a myelin sheath (Fig. 10).

The initial segments of unmyelinated axons of bipolar cells also show a typical undercoating of the axolemma and a fasciculation of microtubules, and these features persist for some 25 \( \mu m \) distal to the site of origin of the axon. None of the axon initial segments of the bipolar cells examined have been found to form axo-axonic synapses. After the initial segments end the axons become thinner and seem to contain only microtubules, vesicles and mitochondria in their cytoplasm (for example, Fig. 12, Ax).

When unmyelinated axons of bipolar cells are impregnated it is seen that the emerging axon soon assumes a vertical orientation, either ascending or descending through the cortex, and forms a number of branches which are also predominantly vertical (Fig. 1). Thus, the orientation of the axons tends to mimic that of the dendritic trees.

In some cases the axons of the bipolar cells form quite intricateplexuses, and examples of such plexuses are those formed by cells c and g (Fig. 1). The ramifications of

Figs. 8–10. Electron micrographs of an unimpregnated bipolar cell with a myelinated axon. In Fig. 8 the cell body is in the lower part of the field and the ascending dendrite (D) gives rise to the axon (Ax). The site of origin of the axon (Ax) is shown at higher magnification in Fig. 9, in which it can be seen that the fasciculation of microtubules (m) of the axon initial segment begins in the dendrite (D). The axon then turns to ascend through the cortex and in Fig. 10 it is shown entering its myelin sheath (M). Fig. 8, \( \times 6000 \); Fig. 9, \( \times 15 000 \); Fig. 10, \( \times 20 000 \).
these axons are shown in more detail in the drawings on the right side of Fig. 11. One of the branches of the axon of cell c divides to produce a vertically oriented complex (arrows) in which the axons have many swellings or boutons, and upon examination by phase microscopy it became apparent that a cluster of apical dendrites of pyramidal neurons passes through this complex. The vertically oriented axonal complex formed by cell g (arrow, Fig. 11) also appears to be closely related to a cluster of pyramidal cell apical dendrites.

The close association between the vertically oriented lengths of unmyelinated axons of bipolar cells and the clusters of apical dendrites of pyramidal neurons is also apparent in thin sections. Indeed, most of the vertically oriented lengths of bipolar cell axons pass parallel to apical dendrites, and their axon terminals are commonly found to form asymmetric synapses with dendritic spines. One such synapse is shown in Fig. 12 in which a labelled axon terminal (At₁) from cell b is synapsing with a stubby spine (s) extending from a dendrite (D). Since this dendrite is contained within a cluster it is probably an apical dendrite. This micrograph also shows two other profiles of labelled axons (At₂ and Ax) which are not synapsing. Except for this one example, however, the profiles of other postsynaptic spines encountered have been separated from their parent dendrites; examples of two of these are given in Figs. 13 and 14. Fig. 13 shows an axonal bouton (At) from cell b forming an asymmetric synapse with two small dendritic spines (s), and in Fig. 14 a labelled axon terminal (At) from cell g is forming an asymmetric synapse with a larger spine (s).

Confirmation of the assumption that some axon terminals of bipolar cells synapse with the spines of apical dendrites of pyramidal neurons has been obtained by

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**Fig. 11.** Camera lucida drawings of axon plexuses formed by bipolar cells. On the left is a drawing of a layer II/III bipolar cell not shown in Fig. 1. The long descending axon of this neuron forms a plexus in layer V. On the right are enlarged drawings of the axons of cells c and g to show details of the plexuses of vertically arranged branches, indicated by arrows.

**Fig. 12.** Electron micrograph of part of the axonal plexus of cell b. The profiles of the axon are labelled by gold particles. The axon is contained within a cluster of pyramidal cell apical dendrites (D) and one terminal (At₁) is synapsing with a spine (s) of one of the dendrites. A second axon terminal (At₂) and a length of axon (Ax) are also present. × 25 000.

**Fig. 13.** Electron micrograph of an axon terminal (At) of cell b synapsing with two dendritic spines (s). × 33 000.

**Fig. 14.** Electron micrograph of an axon terminal (At) of cell g synapsing with a large dendritic spine (s). × 28 000.

**Fig. 15.** Light micrographic montage of a gold-toned Golgi preparation containing a layer III bipolar neuron (B) and a layer V pyramidal cell. The axon of the bipolar cell (Ax) emerges from the ascending dendrite and curves to descend through the cortex (arrows), passing parallel to the apical dendrite (Ap) of the pyramidal cell. × 1200.

**Fig. 16.** Electron micrograph of the preparation shown in Fig. 15. An axon terminal (At) of the bipolar cell is synapsing (arrow) with a dendritic spine (s) of the apical dendrite (Ap). The axon terminal, the spine, and the apical dendrite are all labelled by gold particles. × 35 000.
examination of a gold-toned Golgi preparation in which the descending axon of an impregnated bipolar cell in layer III passes close to, and parallel with, the apical dendrite of an impregnated layer V pyramidal neuron. As shown in Fig. 15, which is a photomontage taken after gold toning and deimpregnation, the axon (Ax) arises from the ascending dendrite of the bipolar neuron (B) and it descends (arrows), passing close to the spines of the apical dendrite (Ap). Upon study of this preparation in the electron microscope two examples of synapses between axon terminals of the bipolar cell and spines of the apical dendrite became evident. One of these is shown in Fig. 16 in which the gold-containing profiles of a bipolar cell axon terminal (At) and a dendritic spine (s) of the apical dendrite (Ap) are forming an asymmetric synaptic junction (arrow). The impregnated dendrite is contained within a cluster of apical dendrites and a number of other synapses involving terminals of the gold-labelled bipolar cell axon and profiles of unlabelled dendritic spines are also present.

While dendritic spines are the neuronal elements most commonly postsynaptic to axon terminals of bipolar cells, a few examples of synapses with shafts of apical dendrites and with dendrites having the characteristics of those from nonpyramidal cells have been encountered. In addition we have an example of a bipolar cell axon terminal forming an asymmetric synapse with the cell body of a nonpyramidal neuron which has the features of a multipolar or bitufted cell (see Peters & Fairén, 1978; Peters & Proskauer, 1980b). Thus, it seems that bipolar cell axon terminals synapse with the dendrites and cell bodies of nonpyramidal cells, as well as with the dendritic spines and shafts of apical dendrites of pyramidal neurons.

Although most of the impregnated axons of bipolar cells have not been seen to extend far from the parent neurons (Fig. 1) we have found one bipolar cell in layer II with a long descending axon. A drawing of this neuron is shown on the left side of Fig. 11. The axon of this neuron originates from the descending primary dendrite and descends, first giving off a recurrent collateral which passes back towards the cell body, and then continuing through the deeper layers of the cortex to reach layer V. Here it branches to form a plexus of vertically oriented collaterals which extend as far as the lower border of layer V. Since this neuron is impregnated by a modified Golgi–Kopsch procedure (Braitenberg et al., 1967) it is not entirely suitable for electron microscopy, but it has been possible to ascertain that most of the terminal branches of the axon in layer V form asymmetrical synapses with dendritic spines. Whether bipolar cells commonly have axons which descend in this fashion to produce a deeper plexus needs further study.

Discussion

The bipolar cells of rat visual cortex constitute a distinct group of nonpyramidal neurons characterized by vertically elongate and narrow dendritic arborizations. Their cell bodies occur throughout layers II/III, IV, and V, and while some bipolar neurons extend from layer II/III to layer V, others are shorter. Like the cell bodies of other nonpyramidal neurons in rat visual cortex which have been examined by electron microscopy (for
example, Parnavelas, et al., 1977; Peters & Fairén, 1978; Peters & Proskauer, 1980a, b; Winfield et al., 1980) those of the bipolar neurons engage in both symmetric and asymmetric synapses. With the possible exception of spinous multipolar neurons (see LeVay, 1973; White, 1978; White & Rock, 1980), this seems to be a common feature of nonpyramidal cells and helps to distinguish them from the pyramidal neurons, which possess only symmetric axo-somatic synapses. In common with other nonpyramidal cells the bipolar neurons also have nuclei with irregular contours and the nuclei of all the bipolar cells we have examined have exhibited a deep, vertically oriented cleft. Moreover the nucleus usually occupies a large proportion of the fusiform cell body, so that except at the upper and lower poles of the cell body the perikaryal cytoplasm is confined to a thin rim. This appearance, together with the presence of generally thin dendrites extending in a vertical direction from the poles of the fusiform cell body, and the presence of few axo-somatic synapses, allows profiles of bipolar cells to be identified in thin sections.

Like their perikarya, the dendrites of bipolar neurons also form both symmetrical and asymmetrical synapses and, as demonstrated, one source of axon terminals forming asymmetrical synapses on neurons with dendrites in layer IV and lower layer III is the lateral geniculate nucleus. A bipolar cell was not included in our earlier study of neurons in rat visual cortex receiving thalamic afferents (Peters et al., 1979), although White (1978), has shown a bipolar cell in mouse Sml cortex to synapse with thalamic axon terminals. This bipolar cell received many thalamic terminals on its cell body, the two primary dendrites, and secondary branches of the ascending primary dendrite, and its dendrites received more thalamocortical synapses per unit length of dendrite than any of the other neurons examined by White (1978). Taken together these studies on the termination of thalamocortical afferents show a sufficiently wide variety of neuronal elements receiving thalamic afferents to warrant the conclusion that any neuronal element contained in layer IV and lower layer III and capable of forming asymmetric synapses is postsynaptic to the thalamic afferents terminating in those layers (also see Peters, 1980; White, 1980). If this is so, then all bipolar cells in area 17 of rat visual cortex probably synapse with thalamic afferents, for they all seem to have at least some portion of their dendritic tree contained within layer IV and lower layer III.

It is common for the axons of bipolar cells to take origin from one of the primary dendrites, and if the axon becomes myelinated, the features of the axon initial segment persist until the axon enters its myelin sheath, but when an axon remains unmyelinated the features persist for about 25 μm before being lost. It is of interest that the axon initial segments of the bipolar cells appear not to synapse with axon terminals. In this respect bipolar cells differ from smooth and sparsely spinous multipolar cells, the axon initial segments of which usually form one or two axo-axonic synapses (Peters & Fairén, 1978), and they are in marked contrast to pyramidal cells for the initial segments of these neurons may have many synapses. This observation agrees with that of Sloper & Powell (1979) who have also remarked that axon initial segments of pyramidal neurons, especially those in upper cortical layers, receive more synapses than the axon initial segments of nonpyramidal neurons.
Axons of bipolar cells frequently form vertically oriented plexuses which pass parallel to apical dendrites and the evidence suggests that many of the asymmetric synapses formed by the bipolar cell axons are with the spines of these dendrites. However, although dendritic spines are the most common postsynaptic element, bipolar cell axon terminals also synapse with shafts of apical dendrites, and with the dendrites and cell bodies of nonpyramidal cells. Thus, the bipolar cell axons synapse with no specific postsynaptic element. In this respect they resemble the smooth and sparsely spinous multipolar neurons in rat visual cortex which form symmetrical synapses with a variety of neuronal elements (Peters & Fairén, 1978; Peters & Proskauer, 1980a). On the other hand, they differ from chandelier cells which seem to be quite specific in forming symmetrical synapses only with the axon initial segments of pyramidal neurons (Somogyi, 1977; Peters, 1980).

The conclusion that the axon terminals of bipolar cells in rat visual cortex form asymmetric synapses differs from that reached by Parnavelas et al. (1977). On the basis of their Golgi-electron microscopic study these authors suggest that the axon terminals of a neuron with the appearance of a bipolar cell, their cell 16, form symmetrical, or type 2 synapses. Their conclusion may be an error attributable to the difficulty in characterizing synapses in material in which junctions are obscured by metallic deposits.

In their Golgi study of rat visual cortex Werner et al. (1979) encountered neurons we would describe as bipolar cells and refer to them as ‘double-bouquet cells’, a name used by Cajal (1911) to describe neurons with tufts of dendrites arising from the upper and lower poles of the cell body. Such neurons have a variety of axonal plexuses, although it is common for their axons to form long, vertically oriented fascicles and Colonnier (1966), Szentágothai (1975), and Jones (1975) express the idea that these fascicles encompass the apical dendrites of pyramidal neurons. In a recent study of such neurons in layer III of cat and monkey visual cortex Somogyi & Cowey (1981) have shown that their axons form symmetric synapses, primarily with small and medium-sized dendritic shafts, and the shafts of pyramidal cell apical dendrites were not encountered amongst the postsynaptic elements. On the other hand, a different type of neuron examined by Somogyi & Cowey (1981), one they refer to as a layer IV spiny double bouquet cell from monkey visual cortex, formed asymmetric synapses with dendritic spines and shafts. Such neurons have very narrow, vertically oriented dendritic fields, and apart from their spines, they bear a strong resemblance to the bipolar cells of rat visual cortex. Previous accounts of bipolar cells in cerebral cortex have been given by Cajal (1911), who shows them in human parietal cortex (his Fig. 373) and cat visual cortex (his Fig. 387), and like those in the rat, these bipolar cells have axons originating from primary dendrites. O'Leary (1941) also describes bipolar neurons in cat visual cortex and shows their axons to form vertical fascicles. Thus, in higher mammals both bipolar and bitufted cells can have axons forming vertically oriented plexuses, and the axons appear to ramify more extensively than those of the bipolar neurons in rat visual cortex.
There seems little doubt that the bipolar cells in rat visual cortex are the neurons which both Loren et al. (1979) and Sims et al. (1980) have shown to combine with antisera to VIP. Comparison between the illustrations of the VIP-immunoactive cells and our drawings of Golgi-impregnated bipolar neurons makes this evident. In addition, both Loren et al. (1979) and Sims et al. (1980) comment that the cell bodies of the VIP-containing neurons occur throughout layers II–V, but while they find the greatest numbers to be in layer II/III (also see Emson & Lindvall, 1979; Fahrenkrug, 1980), in Golgi preparations bipolar cells with cell bodies in layer IV are the ones which most commonly impregnate. It is also pertinent that application of VIP to cortical neurons produces a strong excitation (Phillis et al., 1977) for this would fit with our observation that the axon terminals of bipolar cells form asymmetric synapses, which are generally considered to have an excitatory function.

The role of bipolar cells

While reading this section, reference should be made to Fig. 17 which shows some of the demonstrated synaptic connections of neurons in rat visual cortex.

Geniculocortical afferents (Th. Aff.) terminate in the greatest numbers in lower layer III and layer IV, and both bipolar cells and pyramidal neurons of layers III and V receive axon terminals from this input (Peters et al., 1979). Consequently, these neurons can be expected to be monosynaptically excited by these afferents. Stimulation of a bipolar cell (B₁) could then lead to further excitation of pyramidal neurons (3 and 5) via the asymmetric synapses they form with the axon terminals of the bipolar cell. This would reinforce the initial excitation of the pyramidal cells by the geniculocortical synapses, with the additional disynaptic excitation resulting in a vertical spread of excitation along the clusters of pyramidal neurons (Peters & Walsh, 1972; Feldman & Peters, 1974; Winkelmann et al., 1975). This would lead to a synchronization of activity of the pyramidal neurons. It is tempting to use the expression ‘synchronization along a column of pyramidal neurons’, but as yet a columnar organization has not been demonstrated in visual cortex of the rat and other rodents (see Mangini & Pearlman, 1980). However, columns of neurons exist in cat visual cortex which contains similar bipolar cells (O’Leary, 1941; Regidor & Peters, unpublished data) and the available evidence suggests that, as in the rat, the geniculate afferents to cat visual cortex also terminate on a variety of different neuronal types (for example, Davis & Sterling, 1979; Hornung & Garey, 1980). In cat visual cortex Bullier & Henry (1979) have assessed the ordinal, or serial positions of different types of neurons after stimulation of the optic radiations, and have shown that while some neurons receive monosynaptic input others have a convergence of monosynaptic plus other inputs; yet for other neurons, presumably ones not receiving direct thalamic synapses, the lowest order of excitatory input is disynaptic. At least some of the excitatory convergence may be due to bipolar cells.

Another type of neuron in rat visual cortex is the multipolar neuron with sparsely spinous or smooth dendrites, (S₁ and S₂). The axons of these neurons form symmetrical
Fig. 17. Diagram to illustrate some of the synaptic relationships of bipolar neurons in rat visual cortex. Pyramidal neurons (2, 3 and 5) are stippled. Bipolar cells (B₁ and B₂), and smooth or sparsely spinous multipolar neurons (S₁ and S₂) are black. Axon terminals forming symmetrical synapses are open circles and ones forming asymmetric synapses are filled circles. A discussion of the implications of the synaptic relationships shown in this diagram is presented in the text.
Cortical bipolar neurons

synapses (Peters & Fairén, 1978; Peters & Proskauer, 1980a), and since their terminals label with antibody to glutamic acid decarboxylase (GAD) it can be concluded that these neurons use GABA as their neurotransmitter (Ribak, 1978). Thus they appear to be inhibitory neurons. It was suggested earlier (Peters & Fairén, 1978) that such neurons may be responsible for the inhibition which frequently follows excitation of visual cortical neurons, as demonstrated in cat visual cortex by Toyama et al. (1974). These authors find that some neurons in layers III to V exhibit monosynaptic excitation and disynaptic inhibition which could be produced by a neuronal circuit such as the one on the right of Fig. 17, in which pyramidal neurons (3 and 5) and a sparsely spinous or smooth multipolar cell (S₁) are each monosynaptically excited by geniculocortical afferents, after which the symmetrical synapses between the axon of the multipolar cell with the pyramidal neurons (Peters & Proskauer, 1980a) would produce disynaptic inhibition. Toyama et al. (1974) also show that other neurons, ones in layers II and VI, have a sequence of disynaptic excitation and trisynaptic inhibition, and a neuronal circuit compatible with this sequence is shown on the left of Fig. 17. In this circuit a bipolar cell (B₂) receiving direct thalamic input, synapses with both a layer II pyramidal neuron (2) and a smooth and or sparsely spinous multipolar neuron (S₂) in layer II/III. Excitation of the bipolar cell (B₂) by the geniculocortical afferents would produce disynaptic excitation of both the multipolar cell (S₂) and the pyramidal neuron (2), and trisynaptic inhibition of the layer III pyramidal neuron.

As more information becomes available about the detailed morphology, synaptic connections and neurotransmitters used by different types of neurons in the cerebral cortex, additional neuronal circuits can be deducted to provide morphological explanations for the neurophysiological data being produced. Hopefully, this will ultimately lead to a more comprehensive understanding of how neurons respond to and summate the effects produced by the axon terminals with which they synapse.

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References


LUND, J. S. (1973) Organization of neurons in the visual cortex area 17, of monkey (Macaca mulatta). Journal of Comparative Neurology 147, 455–96.


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PETERS, A. (1980) Two neurons in rat visual cortex with axons forming symmetric synapses. Anatomical Record 196, 146A.


WHITE, E. L. (1980) Thalamocortical synaptic relations: a review with emphasis on the projections of specific thalamic nuclei to the primary sensory areas of the neocortex. *Brain Research Reviews* 1, 275–311.

