Topography of Four Classes of Kenyon Cells in the Mushroom Bodies of the Cockroach

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ABSTRACT

Mushroom bodies (MBs), which are higher centers in the insect brain, are implicated in associative memory and in the control of some behaviors. Intrinsic neurons of the MB, called Kenyon cells, receive synaptic inputs from axon terminals of input neurons in the calyx. Axons of Kenyon cells project into the pedunculus and to the α and β lobes, where they make synaptic connections with dendrites of extrinsic (output) neurons. In this study, we examined the morphology of Kenyon cells in the cockroach by using Golgi stains and found that they can be classified into four classes (K1, K2, K3, and K4), according to the diameter, location, and morphology of the cell bodies, dendrites, and axons. The somata of Kenyon cells of different classes occupy different concentric zones; K1 cells occupy the most central zone, and K4 cells occupy the most peripheral zone. The main processes of Kenyon cells of different classes also occupy different concentric zones in the calyx. Dendrites of K2 and K3 cells are distributed throughout the calycal neuropil, whereas those of K1 and K4 cells cover the outer and inner halves of the depth of the neuropil, respectively. In the pedunculus and the α and β lobes, axons of Kenyon cells of different classes occupy different zones, although the separation is not complete. A class of extrinsic neurons in the α lobe has dendrite-like arbors that cover the zones where either K1, K2, or K3 are located. These neurons probably transmit signals of each class of Kenyon cells. We conclude that, in the cockroach, four classes of Kenyon cells subdivide the cell body region, pedunculus, and lobes of the MBs, whereas subdivision is less prominent in the calycal neuropil. J. Comp. Neurol. 399:162–175, 1998. © 1998 Wiley-Liss, Inc.

Indexing terms: insect; brain; neuroanatomy; associative memory; higher center

Mushroom bodies (MBs) are paired neuropil structures that are located in the center of insect brain. Intrinsic neurons (Kenyon cells) of the MBs extend dendritic arbors into the calyces and receive synaptic input from terminals of input neurons (Steiger, 1967; Schürmann, 1974). The axon of each Kenyon cell runs through the pedunculus and then bifurcates at its base; one extends dorsally in the α lobe, and the other extends medially in the β lobe. In the pedunculus and lobes, Kenyon cells make synaptic connections with dendrites of extrinsic (output) neurons (Frontali and Mancini, 1970; Schürmann, 1970). Axons of extrinsic neurons exit the MB and project to various brain areas (Rybak and Menzel, 1993; Li and Strausfeld, 1997).

MBs have been implicated in associative memory and in some forms of motor control (Huber, 1960; Erber et al., 1980; Mizunami et al., 1993; Davis, 1996). In the honey bee, Erber et al. (1980) noted that local cooling of the MBs results in an impairment of consolidation of olfactory-association memory. In the fruit fly Drosophila, studies using structural and biochemical mutants suggested that MBs play an essential role in olfactory memory (Heisenberg et al., 1985; Nighorn et al., 1991; de Belle and Heizenberg, 1994) and also in courtship behavior (Hall, 1994). In crickets and grasshoppers, electric stimulation evoked various forms of behavior or its elements; thus, it was concluded that MBs are responsible for the selection and coordination of complex behavior patterns (Huber, 1960; Wadepuhl, 1983). In the cockroach, bilateral abla-
Fig. 1. Frontal sections of a cockroach brain that was stained with osmium-ethyl gallate exhibiting the gross morphology of a mushroom body. Posterior (A) and anterior (B) sections were photographed. P, pedunculus; α, α lobe; β, β lobe; MC, medial calyx; LC, lateral calyx. Scale bar = 200 µm.

Fig. 2. Reconstructions of four classes of Kenyon cells (K1, K2, K3, and K4) from frontal sections of Golgi-stained brains. The somata of the K1 cells are located deep within the calycal cup. The somata of the K2 cells are located at the surface of the calycal cup, those of the K3 cells are at the rim of the cup, and those of the K4 cells are at or just outside the rim of the cup. The main processes of four classes of Kenyon cells are arranged concentrically at the base of each calyx: K1 is at the innermost part, and K4 is at the outermost part. NL, neuropil layer; KFL, Kenyon fiber layer; CBL, cell body layer; D, dorsal; V, ventral; L, lateral; M, medial.
Fig. 3. A–F: Morphology of four classes of Kenyon cells observed in the calyx of Golgi-impregnated brains. Frontal sections. The main processes of a pair of K1 cells (A) run along the inner surface of the Kenyon fiber layer (KFL) of the calyx. They extend a few branches into the neuropil layer (NL), each of which forms a small, spherical dendritic field at its tip (arrowheads). A pair of K2 cells (B) extends branches that repeatedly arborize to cover a wide area in the NL. The main process of a K3 cell (C) runs in the outermost part of the KFL. The main process of a K4 cell (D) runs along the NL. In the preparations shown in E and F, a number of Kenyon cells could be identified as K1, K2, K3, or K4 (labelled as 1–4) on the basis of the locations and diameters of the cell bodies and the main processes. Scale bar = 30 µm.
tion of the MBs caused an impairment of navigation based on visual spatial memory (Mizunami et al., 1993). Wire electrode recordings of the activities of extrinsic neurons of the lobes of the MBs of freely behaving cockroaches showed that activities of some neurons precede the initiation of specific motor actions, suggesting that the MBs play a role in the control and possibly in the planning of motor actions (Mizunami et al., 1993). The neural mechanisms underlying these higher functions remain to be clarified.

In the accompanying paper (Mizunami et al., 1998), we report that approximately 15 modular subunits are maintained throughout the output neuropil, i.e., the pedunculus and the $\alpha$ and $\beta$ lobes, of the MBs of the cockroach. Each subunit consists of a pair of dark and light slabs, each of which is formed by the axons of a distinct subset of intrinsic neurons (Kenyon cells). A class of extrinsic neurons of the pedunculus and lobes possesses segmented, dendrite-like arborizations that interact with every other slabs, i.e., with only dark or light slabs; therefore, it was concluded that the slabs are units that transmit output signals from the MBs.

In this study, we examined the morphologies of Kenyon cells in the MBs of the cockroach by using Golgi stains, and we report here that they can be classified into four morphological classes. The soma of four classes of Kenyon cells occupy distinct parts in the cell body region, and their axons also occupy distinct parts in the pedunculus and lobes, but there is a high degree of overlap in the distribution of their dendrites in the calycal neuropil. We conclude that modular subunits in the pedunculus and the lobes are further organized into four larger groups, according to morphological classes of Kenyon cells.

Previous studies using Golgi stains in bees (Mobbs, 1982), ants (Goll, 1967), flies (Strausfeld, 1976), moths (Pearson, 1971), and crickets (Schürmann, 1973) showed that Kenyon cells can be classified into a few morphological types and that these types often subdivide the whole or a part of the MBs. The present findings on cockroaches are compared with those noted for other insects in order to discuss general and specific features of the organization of insect MBs.

MATERIALS AND METHODS

Adult male and female cockroaches (Periplaneta americana) from a colony raised in our laboratory were used. The cockroaches were maintained at 25–27°C and in 12 hour light/12 hour dark conditions. Each cockroach was anesthetized with cooling on ice, and the head was removed and mounted on a small dish. Impregnations were carried out according to the Golgi/Colonnier and mixed Golgi rapid/Colonnier methods (Strausfeld, 1980), the details of which are described in the accompanying paper (Mizunami et al., 1998). Golgi-impregnated brains were dehydrated and embedded in soft Araldite and sectioned at 50–110 µm.

The osmium-ethyl gallate procedure was followed according to Wigglesworth (1957). Isolated heads were mounted in a dish. The head capsules were opened and immersed in cacodylate-buffered 2% glutaraldehyde and 1% paraformaldehyde for 24 hours. The brains were then dissected out and osmicated in 1% osmium tetroxide for 1–2 hours at 4°C. The tissues were washed in buffer and then transferred to 0.5% ethyl gallate for 2 hours at 4°C. The specimens were dehydrated, embedded in soft Araldite, and sectioned at 50–110 µm. The osmium-ethyl gallate procedure was followed according to Wigglesworth (1957). Isolated heads were mounted in a dish. The head capsules were opened and immersed in cacodylate-buffered 2% glutaraldehyde and 1% paraformaldehyde for 24 hours. The brains were then dissected out and osmicated in 1% osmium tetroxide for 1–2 hours at 4°C. The tissues were washed in buffer and then transferred to 0.5% ethyl gallate for 2 hours at 4°C. The specimens were dehydrated, embedded in soft Araldite, and sectioned at 50–110 µm. For reduced silver staining, a variation of Otsuka (1962) and the original methods of Bodian impregnation (Bodian, 1936) were used for 10–12 µm paraffin sections. Preparations were observed under real time three dimensions (Edge Scientific Instruments, Santa Monica, CA), Nomarski interference contrast (Olympus, Tokyo, Japan), and conventional microscopes. Observations at 1,000× were made by using an oil-immersion objective lens. Drawings were made with the aid of a camera lucida.

RESULTS

We obtained approximately 250 brains in which Kenyon cells had been impregnated successfully by using Golgi procedures. In about 50% of these brains, profiles of
individual Kenyon cells could be traced for their whole extent in serial sections. In the remaining brains, such large numbers of Kenyon cells were stained that it was not feasible to trace individual cells. By observing the former preparations, we classified Kenyon cells into four types (K1, K2, K3, and K4) on the basis of 1) the diameter and position of the cell bodies, 2) the diameter and the position of the main processes in the calyx, and 3) the morphology of dendritic arborizations. Axons of the Kenyon cells of different types occupy different zones in the pedunculus and lobes, although the segregation is not complete. The presence of four types of Kenyon cells was also evident in the latter mass-impregnated brains. Here, we first describe the morphology of four classes of Kenyon cells in the

![Fig. 5](image)

**Fig. 5.** A–E: Serial horizontal sections in which axons of K1 cells originating from various parts of the medial calyx are traced to the α lobe. Axons of K1 cells converge at the base of the calyx (A). Axon bundle of K1 cells from the medial calyx (MC) and that from the lateral calyx are arranged side by side at the head of the pedunculus (P; B, C) and occupy the most medial part in the pedunculus (C, D). In B, M and L indicate axons of Kenyon cells derived from the medial and lateral calyces, respectively. They continue to the most posterior part in the α lobe (D, E). Anterior is at the top, and lateral is on the right. Scale bar = 50 µm.
neuropil, is filled with numerous microglomeruli, where we refer to as the neuropil layer (NL) or calycal
continues to the pedunculus (Fig. 1A). The outer layer, which we refer to as the neuropil layer (NL) or calycal neuropil, is filled with numerous microglomeruli, where axon terminals of input neurons make synaptic contacts with dendrites of Kenyon cells (Schürmann, 1974; Weiss, 1974).

**Morphology of four classes of Kenyon cells in the calyx**

Each MB of the cockroach contains a lateral and a medial calyx (Fig. 1A). Each calyx can be divided into inner and outer layers (Sanchez, 1933; Weiss, 1974). The inner layer, which we refer to as the Kenyon fiber layer (KFL), consists of a large number of main processes of Kenyon cells running in parallel (Figs. 1, 2). Ventrally, the KFL continues to the pedunculus (Fig. 1A). The outer layer, which we refer to as the neuropil layer (NL) or calycal neuropil, is filled with numerous microglomeruli, where axon terminals of input neurons make synaptic contacts with dendrites of Kenyon cells (Schürmann, 1974; Weiss, 1974).

**K1 cells.** The soma of K1 cells are 6–8 µm in diameter and fill the bottom half of the calycal cups (Figs. 2, 3A,E,F). The main processes originating from the cell bodies enter the inner surface of the KFL. Immediately after entering the KFL, some K1 cells give off side branches, which are connected to one another at the inner surface of the KFL (Fig. 4A). The main processes of K1 cells run in the innermost part of the KFL (Figs. 2, 4B). Each K1 cell extends three to five processes into the NL, each giving rise to several short arborizations at its tip to form a spherical dendritic field, the typical diameter of which is 15–30 µm (Fig. 2). Each dendritic field appears to cover a single microglomerulus. The dendrites are rich in spines.

Observations of a large number of K1 cells have shown that dendrites of K1 cells are distributed in all radial and concentric zones of the calycal neuropil (see Fig. 5A). Dendritic arbors of K1 cells are distributed more densely in the outer half of the depth of the neuropil (Figs. 2, 3A).

**K2 cells.** The soma of K2 cells are located dorsal to the K1 cells and fill a superficial area within the calycal cups (Figs. 2, 3E,F). The diameters of the somata are 6–8 µm, similar to those of K1 somata. Their main processes run outside those of K1, in the central zone of the KFL (Figs. 2, 3A). Each K2 cell extends several branches in the NL, which send off arborizations to sparsely cover a large number of microglomeruli (Figs. 2, 3B). The arborizations exhibit numerous spines.

Observations of K2 cells in a number of Golgi-impregnated brains have shown that dendrites of K2 cells are distributed in all radial and concentric zones of the calycal neuropil (see Fig. 7A). They also are distributed almost evenly throughout the depth of the neuropil.

**K3 cells.** The cell bodies of K3 cells are slightly larger (7–9 µm in diameter) than those of K1 or K2 cells (Figs. 2, 3C,E,F), and they are located peripheral to those of K2, at the rim of the calycal cups. The main processes of K3 cells, which appeared thicker than those of K1 and K2 cells, enter the KFL and run in the outermost part of the KFL (Fig. 2). Each process gives off several branches in the NL, which arborize to sparsely cover a large number of microglomeruli. The arborizations of K3 cells are rich in spines. It was often difficult to distinguish K2 and K3 cells from each other by diameter or positioning of their cell bodies, main processes, and axons.

Observations of a number of preparations have shown that dendrites of K3 cells cover the whole radial and concentric zones of the calycal neuropil (see Fig. 5A). They also are distributed almost evenly throughout the thickness of the neuropil.

**K4 cells.** The soma of K4 cells are 8–10 µm in diameter, the largest among the four classes, and they are located peripheral to those of K3 cells, at or just outside the rim of calycal cups (Figs. 2, 3D–F). The number of K4 cells impregnated in each MB is less than ten, which is at least ten times fewer than those of other classes. The main processes of K4 cells are the thickest among the four classes. Most K4 cells run in the NL and give off short collaterals of 10–30 µm in various directions in the KFL. Their dendritic arbors are distributed as discrete patches within a cylinder, each of which appears to cover a microglomerulus (Figs. 2, 3D). The dendrites exhibit characteristic clawed endings (Fig. 3D). Some K4 cells run in the outermost part of the KFL and extend several branches with clawed endings into the NL.

Observations of a number of preparations have shown that dendritic arborizations of K4 cells are confined mostly to the inner half of the depth of the NL and that they are distributed more densely in the most peripheral concentric zone than in the middle or central concentric zones of the NL (Figs. 2, 3D). Their dendrites are distributed in all radial areas of the NL.

**Projections of axons of four classes of Kenyon cells from the calyx to the lobes**

The axon of each Kenyon cell descends anteroventrally through the pedunculus and then bifurcates to form the
Observations of reduced silver preparations have shown that the pedunculus and $\alpha$ lobes consist of approximately 15 modular subunits (M1–M15). The location of each modular subunit in the $\alpha$ lobe is reported in the accompanying paper (Mizunami et al., 1998). Here, the positions of axons of four classes of Kenyon cells in the $\alpha$ lobe have been mapped into the positions of modular subunits in order to estimate the relationship between the modular subunits (M1–M15) and the four morphological classes of Kenyon cells (K1–K4).

**K1 cells.** In the pedunculus and lobes, K1 cells are often seen as a group of 40–100 axons, either as a tightly packed axonal mass (Figs. 4, 5) or as a thin sheet. The latter is described in the accompanying paper (Mizunami et al., 1998). In the preparation shown in Figure 5, a number of K1 cells with dendrites that are located in various parts of the medial calyx are traced to the $\alpha$ lobe through the pedunculus in serial horizontal sections. Axons of K1 cells converge at the base of the calyx (Fig. 5A) and form a very tightly packed bundle (Figs. 4B, 5B). K1 axons of the medial calyx run side by side with those of the lateral calyx (Fig. 5B,C) and occupy the most medial part in the pedunculus (Figs. 4B, 5D). The axons occupy the most posterior part in the $\alpha$ lobe (Fig. 5D,E) and the $\beta$ lobe (not shown).

The distribution of K1 cells in relation to modular subunits (M1–M15) was examined in the $\alpha$ lobe. Studies were made on 23 preparations in which more than 20 cells were identified as K1 cells from their dendritic morphology in the calyx and traced to the $\alpha$ lobe. At least one K1 cell was found in the area corresponding to M1 in all 23 preparations, at least one K1 cell was found in M2 in 16 (70%) preparations, one was found in M3 in six (26%) preparations; and one was found in M4 in one (4%) preparation (Fig. 6). There was no preparation in which K1 cells were positioned in M5–M15. To facilitate description, we define the typical distribution area of a class of Kenyon cells as the area in which at least one cell was located in more than one in three of the preparations examined. Following this definition, the typical distribution area of K1 cells is M1 and M2.

**K2 cells.** Typically, K2 cells are seen as a group of 20–80 cells, either as a population of axons that are dispersed sparsely over a wide area (Fig. 7B,C) or as a thin sheet in the pedunculus and lobes. In Figure 7, axons of a population of K2 cells originating from various areas of the medial calyx are traced to the pedunculus and the $\alpha$ lobe in serial horizontal sections. These axons converge at the base of the calyx (Fig. 7A) and occupy a central zone in the bundle of Kenyon cell axons from the medial calyx.
Axons of K2 cells from the medial calyx are arranged side by side with those from the lateral calyx and then are mixed together as they project to the lobes. Axons of K2 cells occupy the central zone in the \( \alpha \) lobe (Fig. 7C) and the \( \beta \) lobe (not shown).

Even without a quantitative study, it was obvious that the distribution area of K2 cells includes M4–M12. Thus, our examination of the distribution of K2 cells was confined to areas corresponding to M1–M3 and M13–M15. For the former, we selected 17 preparations in which more than 15 cells were identified as K2 cells, based on their morphology in the calyx, and traced them to the area corresponding to M1–M3 in the \( \alpha \) lobe. There was at least one K2 cell in the area corresponding to M1 in all 17 preparations, at least one in M2 in 14 (83%) preparations, and at least one in M1 in four (18%) preparations (Fig. 6).

For the latter, we selected 20 preparations in which more than ten K2 cells were located in the area corresponding to M13–M15 in the \( \alpha \) lobe. At least one K2 cell was located in the area corresponding to M13 in all 20 examined preparations, and at least one was located in M14 in ten (50%) preparations, but no K2 cells were found in the area

Fig. 8. A–D: K3 cells of the medial calyx are traced to the \( \alpha \) lobe in serial horizontal sections. Axons of K3 cells of the medial calyx (MC) converge at its base (A) and occupy the lateral area (areas surrounded by dotted lines in B and C) in the pedunculus (P). They run side by side with K3 cells of the lateral calyx (LC) and then are mixed together and occupy the anterior area in the \( \alpha \) lobe (areas surrounded by dotted lines in C and D). In B, M and L indicate axons of Kenyon cells of the medial and lateral calyces, respectively. Anterior is at the top, and lateral is to the right. Scale bar = 50 µm.
corresponding to M15 in any preparation (Fig. 6). Thus, we conclude that the typical distribution area of K2 cells is M2–M14.

K3 cells. In a number of Golgi preparations, 30–100 axons of K3 cells either were distributed densely in a narrow area, or they formed a thin sheet in the pedunculus (Fig. 8B–D). In Figure 8, axons of a number of K3 cells derived from various parts of the medial calyx are traced to the head of the pedunculus (Ped). Axons of K2 cells project centrally, and those of K3 cells run along the periphery to reach the lateral part. Some K3 axons take an anterior route (A), whereas others take a posterior route (P) to reach the lateral part. Dorsal is at the top in A and B, and anterior is at the top in C and D. Lateral is to the right in A–D. Scale bars = 100 µm in A,B, 30 µm in C.

Fig. 9. Some morphological features of K3 cells compared with K1 and K2 cells. A,B: Two serial frontal sections showing that axons of K1, K2, and K3 cells occupy separate areas in the pedunculus. The main processes of K1, K2, and K3 cells occupy the innermost, central, and outermost parts of the Kenyon fiber layer (A), and their axons occupy the medial, central, and lateral parts of the pedunculus, respectively (B). C,D: A photomicrograph of a horizontal section (C) and its reconstruction (D) showing that axons of K2 and K3 cells from the medial part of the medial calyx (MC) run along different routes at the head of the pedunculus (Ped). Axons of K2 cells project centrally, and those of K3 cells run along the periphery to reach the lateral part. Some K3 axons take an anterior route (A), whereas others take a posterior route (P) to reach the lateral part. Dorsal is at the top in A and B, and anterior is at the top in C and D. Lateral is to the right in A–D. Scale bars = 100 µm in A,B, 30 µm in C.
mixed together as they project to the lobes. They run in the anterior part of the α lobe (Fig. 8C) and the β lobe (not shown).

Separate distributions of axons of K1, K2, and K3 cells in the pedunculus are seen in serial frontal sections of a Golgi preparation in Figure 9A,B, in which densely packed axons of K1 cells occupy the medial edge, K2 cells are distributed sparsely at the center, and dense axons of K3 cells occupy the lateral edge of the pedunculus (Fig. 9B).

At the head of the pedunculus, axons of K3 cells originating from the medial area of the medial calyx (Fig. 9C,D) and the lateral calyx (not shown) take a distinct route from those of K2 cells that originate from the same area. Whereas axons of K2 cells run straight to the center of the pedunculus, axons of K3 cells take a detour route along the periphery of the pedunculus until they reach the lateral parts. This separation provides convincing evidence that K2 and K3 cells are of separate morphological classes. Note that the axons of some K3 cells take an anterior route, whereas the others take a posterior route to reach the lateral part (Fig. 9C,D). The separation of K3 axons indicates that the transformation of concentric arrangements of main processes of four classes of Kenyon cells at the base of the calyx into a linear arrangement in the pedunculus requires a break point, which is located medially (see Discussion).

Among 19 preparations in which more than 20 cells were identified as K3 cells based on their morphology in the calyx and were traced to the α lobe, at least one K3 cell was seen in the area corresponding to M15 in seven (37%) preparations, one was seen in M14 in all 19 preparations, one was seen in M13 in eight (42%) preparations, and one was seen in M12 in two (10%) preparations (Fig. 6). K3 cells were never observed in the area corresponding to M1–M11 in any preparation. We conclude that the typical distribution area of K3 is M13–M15.

**K4 cells.** Fewer than ten K4 cells were seen in each MB. Axonal sheets formed by K4 cells were never observed. Figure 10 shows a K4 cell that was reconstructed from serial horizontal sections from the medial calyx to the base of the pedunculus. The cell body is not impregnated. The axon projects to the most lateral part of the pedunculus and continues to the most anterior parts of the α and β lobes (not shown). Observations of ten preparations in which one to eight K4 cells were traced to the α lobe show that all K4 axons are located in the area corresponding to M15 (Fig. 6) in either the M15 light slab or the M15 dark slab.

**Morphology of four classes of Kenyon cells in the pedunculus and lobes**

Axons of K1 and K2 cells appear to be the thinnest, and those of K4 cells appear to be the thickest among the four classes throughout the pedunculus and the lobes. Axons of all classes of Kenyon cells exhibit varicosities in the α and β lobes. Axons of K3 and K4 cells exhibit numerous spines in addition to varicosities throughout the α lobe (Fig. 11) and the β lobe (not shown). This is in contrast to K1 and K2 cells, which exhibit spines only rarely. In the pedunculus, axons of all classes of Kenyon cells are smooth and only rarely have varicosities or spines.

**Dendritic morphology of a class of extrinsic neurons of the α lobe**

An extrinsic neuron of the α lobe is shown in Figure 12A. It has three sets of dense, dendrite-like, spiny arborizations that appear to cover three neighboring slabs, namely, M15 light slab (M15L), M14 dark slab (M14D), and M14L: One densely covers the entire region of M15L, the other densely covers the whole M14L, and the remaining one covers a part of the thickness of M14L (Fig. 12B, arrowheads). Because these areas match the distribution area of K3 cells, it is likely that this neuron receives synaptic inputs mainly from K3 cells. The extrinsic neuron depicted in Figure 12C has segmented, dendrite-like arbors located in an area roughly corresponding to M4–M12. Because this area is formed almost exclusively by K2 cells, the neuron probably receives input from K2 cells. The segmentation of arborizations indicates that the neuron interacts with every other slabs, i.e., only either dark or light slabs (Mizunami et al., 1997). The extrinsic neuron depicted in Figure 12D extends presumed dendrites in an area roughly corresponding to M1–M8, the density of which is very high in M1–M3, where K1 cells are distributed, and much lower in M4–M8, which is formed by K2 cells. This neuron appears to receive input mainly from K1 cells, with some additional input from K2 cells. These observations suggest that there is a class of extrinsic neurons that specifically or mainly transmits signals of each of the K1–K3 cells. We have not yet observed extrinsic neurons, the dendrites of which are confined to M15, where K4 cells are distributed.

**DISCUSSION**

We find that Kenyon cells of the cockroach MBs can be classified into four classes (K1, K2, K3, and K4), according to the position, diameter, and morphology of the cell bodies, dendrites, and axons. The positions of the four
classes of Kenyon cells are summarized in Figure 13. These four classes differ in several independent factors; thus, they represent separate morphological classes rather than mere variations within a single class. Observations of Golgi-stained neurons usually do not allow for estimation of the relative number of each class of neurons, because Golgi staining often exhibits a prominent selectivity (Strausfeld, 1980). However, because the area occupied by each class in the α lobe is K2 > K3 > K1 > K4, it is most probable that the number of the four classes follows the same sequence. Here, we regard K2 and K3 cells as the majority classes and K1 and K4 cells as minority classes.

According to dendritic morphology, the four classes are grouped into three: K1 cells exhibit small-field, spiny dendrites; K2 and K3 cells exhibit large-field, spiny dendrites; and K4 cells exhibit small-field, clawed dendrites. Kenyon cells with spiny and clawed dendrites have been observed in flies (Strausfeld, 1976), bees (Mobbs, 1982), moths (Pearson, 1971), and crickets (Schürmann, 1973). It would be interesting to investigate whether dendrites with spiny and clawed endings form different types of microglomeruli and make synaptic contact with terminals of different types of input neurons.

Some K1 cells give off side branches, which are tightly intermingled at the inner surface of the KFL. Note that these branches are located far from the terminals of input neurons that are distributed in the outer neuropil layer. We speculate that these K1 cells form reciprocal synaptic connections. Connections among dendrites of Kenyon cells in the calyx have not been noted in any other insects. In the locust MB, Kenyon cells appear to make reciprocal, excitatory connections in the pedunculus to synchronize activities, and the possible functional significance of the synchronized electrical activity has been discussed (Laurent and Naraghi, 1994).

The somata of Kenyon cells of different classes occupy different concentric zones in the cell body region; K1 cells form the most central zone, and K4 cells form the most peripheral zone (Fig. 13). Somata at the central zone (K1 and K2 cells) are smallest, and those at the most peripheral zone (K4 cells) are the largest. This observation agrees with previous findings in the cockroaches Blatta orientalis (Sanchez, 1933) and Periplaneta americana (Weiss, 1974), in which somata at the peripheral zone appear larger than those at the central zone. Weiss also noted in his reduced silver study that a class of Kenyon cells with large somata near the calycal rim tangentially enter the outer neuropil layer of the calyx and that their axons occupy the peripheral rim at the head of the pedunculus. These cells are apparently the same as K4 cells.

The main processes of four classes of Kenyon cells are arranged concentrically at the bases of calyces and are converted into a quasilinear arrangement of flattened layers at the pedunculus and lobes (Fig. 13). This is similar to the findings by Mobbs (1982) in the honey bee, in which the calyces are separated into three concentric zones. The concentric arrangement of Kenyon cells in the calyces is transformed to a linear arrangement in the pedunculus, which continues to the lobes. This transformation requires a break point, which is located medially in the cockroach, as discussed above for K3 cells (Fig. 9C,D); but is located posteriorly in the honey bee (Mobbs, 1982). The ant, like the bee, has its calyces organized in a series of concentric neuropil compartments; however, in the α and β lobes, the polar organization is maintained rather than being transformed into a Cartesian organization (Goll, 1967). In the cricket, Schürmann (1973) noted a maintenance of the calycal Kenyon cell groupings in the pedunculus and the α lobe. In the fruit fly Drosophila, there is a subdivision according to biochemical types of Kenyon cells (Yang et al.,

![Fig. 11. Morphologies of axons of K1 (A), K2 (B), K3 (C), and K4 (D) cells in frontal sections of the α lobe. Axons of K1 and K2 cells exhibit varicosities. Axons of K3 and K4 cells are rich in spines (arrowheads) as well as varicosities. Axons of K4 cells appear to be the thickest, and axons of K1 and K2 cells appear to be the thinnest among the four cell types. Scale bar = 20 µm.](image-url)
1995), and ablation of a specific type of Kenyon cell results in a defect in sexual behavior, whereas that of other types does not (O’Dell et al., 1995).

In the α and β lobes of the MBs of the cockroach, axons of K1 and K2 cells exhibited numerous varicosities, whereas those of K3 and K4 cells exhibited numerous spines as well as varicosities. Because varicosities are interpreted as presynaptic, and spines are considered to be postsynaptic (Strausfeld, 1976), it is likely that K3 and K4 cells are both pre- and postsynaptic. Frontali and Mancini (1970) noted in their electron microscopic studies of the cockroach α lobe that some Kenyon cells make synaptic connections not only with extrinsic neurons but also with other Kenyon cells. Li and Strausfeld (1997) noted that some neurons of the lobes had varicose arborizations and considered these neurons as input neurons to the lobes. It would be interesting to investigate whether presynaptic elements of K3 and K4 cells are Kenyon cells, or input neurons, or both.

Although there is a clear subdivision according to morphological classes of Kenyon cells in the cell body region,
pedunculus, and lobes, subdivision of calycal neuropil according to dendrites of four classes of Kenyon cells is less prominent. It is evident that K1 and K4 cells, the minority types, subdivide the neuropil: Dendrites of K1 and K4 cells are distributed more densely in the outer and inner halves of the depth of the neuropil, respectively. Dendrites of K2 and K3 cells, the majority types, however, are distributed more or less evenly throughout the neuropil, which obscures the subdivision formed by minority types. This differs from reports for MBs of bees (Mobbs, 1982) and ants (Goll, 1967), in which there is a prominent subdivision of the calycal neuropil according to morphological types of Kenyon cells. In bees, for example, there are three concentric divisions in the calycal neuropil that are related to sensory modalities of input neurons: The olfactory input goes to the outermost part (lip), the visual input goes to the median part (collar), and both olfactory and visual input and, possibly, mechanosensory inputs go to the central part (basal ring; Mobbs, 1982).

Recently, Nishikawa et al. (1998) found a subdivision in the calycal neuropil of the MB of the cockroach according to dendrites of different types of input neurons: Some deutocerebral and protocerebral input neurons terminate in specific concentric zones in the calycal neuropil. Thus, it is likely that different subsets of Kenyon cells extend dendrites in different concentric zones and receive synaptic input from terminals of different types of input neurons. Nishikawa et al. also found that visual input neurons extend terminal arbors in the inner layer of the calycal neuropil. It is interesting to note that dendrites of K4 cells are almost confined within this layer. In addition, there is a class of GABA-immunoreactive, multimodal input neurons that has been found to extend terminal arborizations in the entire calycal neuropil (Nishino and Mizunami, 1998; Yamazaki et al., 1998). The patterns of distribution of dendrites of individual Kenyon cells of each class need to be studied to clarify further the functional subdivisions of the calycal neuropil in the MB of the cockroach.

We conclude that there are at least two distinct types of structural modules in the MB of the cockroach. The first consists of approximately 15 repetitive modular subunits, which are described in the accompanying paper (Mizunami et al., 1998). The second consists of four subdivisions according to morphological classes of Kenyon cells. It would be interesting to clarify whether these structural modules act as functional modules, like the functional columns in the mammalian cerebral cortex (Hubel and Wiesel, 1974; Fujita et al., 1992).

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LITERATURE CITED


