AXO-AXONIC SEPTATE JUNCTIONS IN THE BASKET FORMATIONS
OF THE CAT CEREBELLAR CORTEX

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INTRODUCTION

In the cat cerebellar cortex, as in most higher vertebrates, the descending branches of the basket cell axon envelop the Purkinje cell body in the region of its axon hillock in a basket-like arborization (Ramón y Cajal, 1909). This report further characterizes the basket formations of the cat and describes septate junctions between the descending branches of the basket cell axons and between some of these branches and the initial segment of the Purkinje cell axon.

Since their original description in *Hydra* (Wood, 1959), septate junctions have been found between many different kinds of invertebrate epithelial cells (Wiener et al., 1964; Locke, 1965; Danilova et al., 1969; Satir and Gilula, 1970, among others) and between some vertebrate epithelial cells (Potts, 1966; Barros and Franklin, 1968; Rupec and Braun-Falco, 1968) although they have not been reported previously in the central nervous system. Structures resembling septate junctions have been described in the turtle retina (Lasanky, 1969).

MATERIALS AND METHODS

Brains of adult cats were fixed by vascular perfusion with either potassium dichromate–buffered osmium tetroxide (Gobel and Dubner, 1969) (see Fig. 3) or with a sodium cacodylate–buffered glutaraldehyde-paraformaldehyde solution (Karnovsky, 1965) (see Figs. 1, 2, 4–7). Specimens were postfixed overnight in solutions of 1% osmium tetroxide in the same buffer system used in the perfusate. Calcium chloride (0.2%) was included in all fixatives. Specimens perfused with aldehydes were also block-stained in 0.5% uranyl acetate (Karnovsky, 1967). After dehydration in a graded series of alcohols and embedding in Maraglas (Erlandson, 1964), thin sections were cut from samples of the flocculus and vermal and hemi-

spheric portions of the anterior and posterior lobes on an LKB Ultrotome I, stained with lead citrate (Reynolds, 1963), and examined in a Siemens Elmiskop I at 80 kv.

RESULTS AND DISCUSSION

Recent electron microscope studies have shown that the proximal segments of the descending branches of the basket cell axon form numerous synaptic contacts on the Purkinje cell body (Hámori and Szentágothai, 1965; Fox et al., 1967) and that the terminal or distal segments of these branches form a dense tangle around the axon hillock and initial segment of the Purkinje cell axon (Palay, 1964; Hámori and Szentágothai, 1965). In the cat, three to seven distal segments tend to aggregate in small clusters and the distal segments, in turn, emit numerous thin finger-like processes which collect in small pools (Fig. 1). The clusters of distal segments are separated from each other by the finger-like processes and astrocytic processes (Fig. 1).

A distal segment usually contains several large mitochondria with extremely dense matrices, neurofilaments, a few microtubules, profiles of agranular reticulum, occasional multivesicular bodies, and granular vesicles, as well as spherical and flattened synaptic vesicles (Fig. 1). Distal segments rich in neurofilaments contain few synaptic vesicles (Figs. 1 and 5). The finger-like processes usually contain a few synaptic vesicles and an occasional multivesicular body or mitochondrion.

At many places between adjacent distal segments regularly spaced septa traverse the intercellular space (Fig. 2). Such areas resemble septate junctions which have been described in many invertebrate tissues (Wood, 1959; Wiener et al., 1964; Locke, 1965) with respect to the following
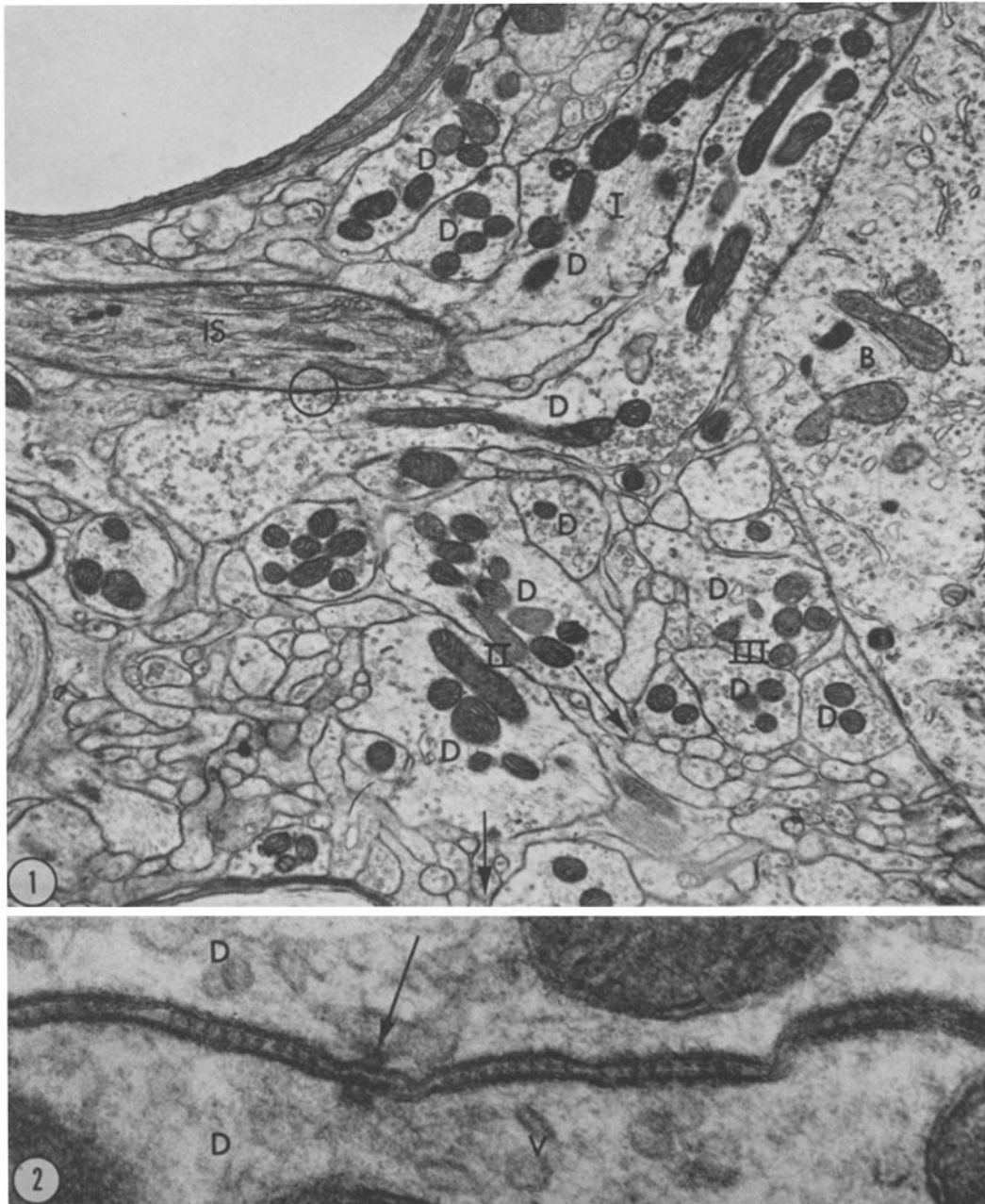
FIGURE 1   Initial axonal segment (*IS*) and lower pole of a Purkinje cell body (*B*). The basket formation is composed of the numerous distal segments (*D*) of the descending collaterals of the basket cell axon which are grouped in small clusters (I, II, III). The distal segments give rise to finger-like projections (arrows) which are often aggregated in small pools (lower left). One distal segment which synapses on the cell body also forms a short synapse (circle) on the initial segment. × 13,800.

FIGURE 2   A series of short septate junctions between two distal segments (*D*). A short desmosome-like structure (arrow) separates two of the septate junctions. Synaptic vesicles (*V*). × 134,500.

four parameters: septal thickness, interseptal spacing, width of the intercellular space, and the center-to-center spacing of the septal subunits (Gilula et al., 1970) which are seen in tangential sections through the junctions. In view of these similarities these axo-axonic specializations will be designated as septate junctions.

Each distal segment within a cluster is joined to its neighbors by one or more septate junctions (Figs. 2 and 5). Septate junctions are also found between distal segments and finger-like processes (Fig. 5) and between finger-like processes (Fig. 4). An occasional finger-like process is attached to the initial segment of a Purkinje cell axon by a septate junction (Fig. 3).

Most septate junctions are 0.15–0.5 μ long (Fig. 2) although a few may attain lengths of 0.7 μ. Others, particularly those between finger-like processes, are quite small and consist of only three or four septa (Fig. 3). Although a few synaptic vesicles are found in the vicinity of some junctions (Fig. 3), vesicle clusters and membrane densities are conspicuously absent from junctional areas (Figs. 2, 4, and 5).

The septate junctions exhibit similar characteristics irrespective of the kinds of axonal processes they join. The cell membranes in the junctional areas assume a parallel relationship, with the intercellular distance ranging between 110 and 180 A. The septa are 50–80 A thick; their center-to-center spacing varies from 160 to 220 A. In specimens block-stained in uranyl acetate the centers of the cross-sectioned septa often stain more intensely than the sides, giving the septa a circular

appearance (Fig. 7). When the section plane passes through the junction approximately parallel to the cell membranes, the longitudinally sectioned septa have a honeycombed appearance in which each septum appears as a folded or wavy sheet (Fig. 5). The wave forms of adjacent septa are usually somewhat out of phase, giving the appearance of tightly packed oval or flattened hexagonal subunits (Fig. 6). These subunits have a center-to-center spacing of approximately 220 A which is more than twice the distance between the subunits of gap junctions (Revel and Karnovsky, 1967). This honeycombed appearance resembles that described in the septate junctions of several invertebrate tissues (Locke, 1965; Danilova et al., 1969; Gilula and Satir, 1970). At the periphery of the septate junctions between distal segments, the intercellular space narrows down to 20–30 A for short intervals (Figs. 5 and 7). Many of the septate junctions involving finger-like processes or those located where adjacent distal segments diverge from each other terminate without such focal constrictions (Fig. 3). Extensive membrane appositions or gap junctions have not been seen. Desmosomes, or puncta adhaerentia, are often found between the basket cell axons and commonly lie adjacent to septate junctions (Fig. 2). It is not uncommon for a desmosome to join a basket cell axon and an astrocytic process.

In their structure the axo-axonic septate junctions, while generally similar to the septate junctions found in many invertebrate epithelial tissues, differ from them in several respects. Most notable are their short length, their occurrence in short

FIGURE 3 A distal segment (D) emits a finger-like projection (F) which forms a septate junction (vertical arrow) on the initial axonal segment of a Purkinje cell (IS). The axolemmal undercoating is absent where the finger abuts on the initial segment. A second very short septate junction is seen at the oblique arrow. Microtubule bundles (T). Astrocytic processes (A). × 67,500.

FIGURE 4 Two finger-like processes are joined by a septate junction. The septa are inclined at an angle of 60° with respect to the cell membranes. × 60,000.

FIGURE 5 A distal segment (D) is joined to three adjacent ones ($D1$, $D2$, $D3$) by septate junctions. The septa present a honeycombed appearance (arrows) when the junctions are sectioned parallel to the cell membranes. Finger-like process (F). Astrocytic process (A). × 45,200.

FIGURE 6 At higher magnification individual units of the honeycomb appear as tightly packed ovals or flattened hexagons. × 180,000.

FIGURE 7 The intercellular space is constricted at the periphery of a septate junction between two distal segments. × 240,000.
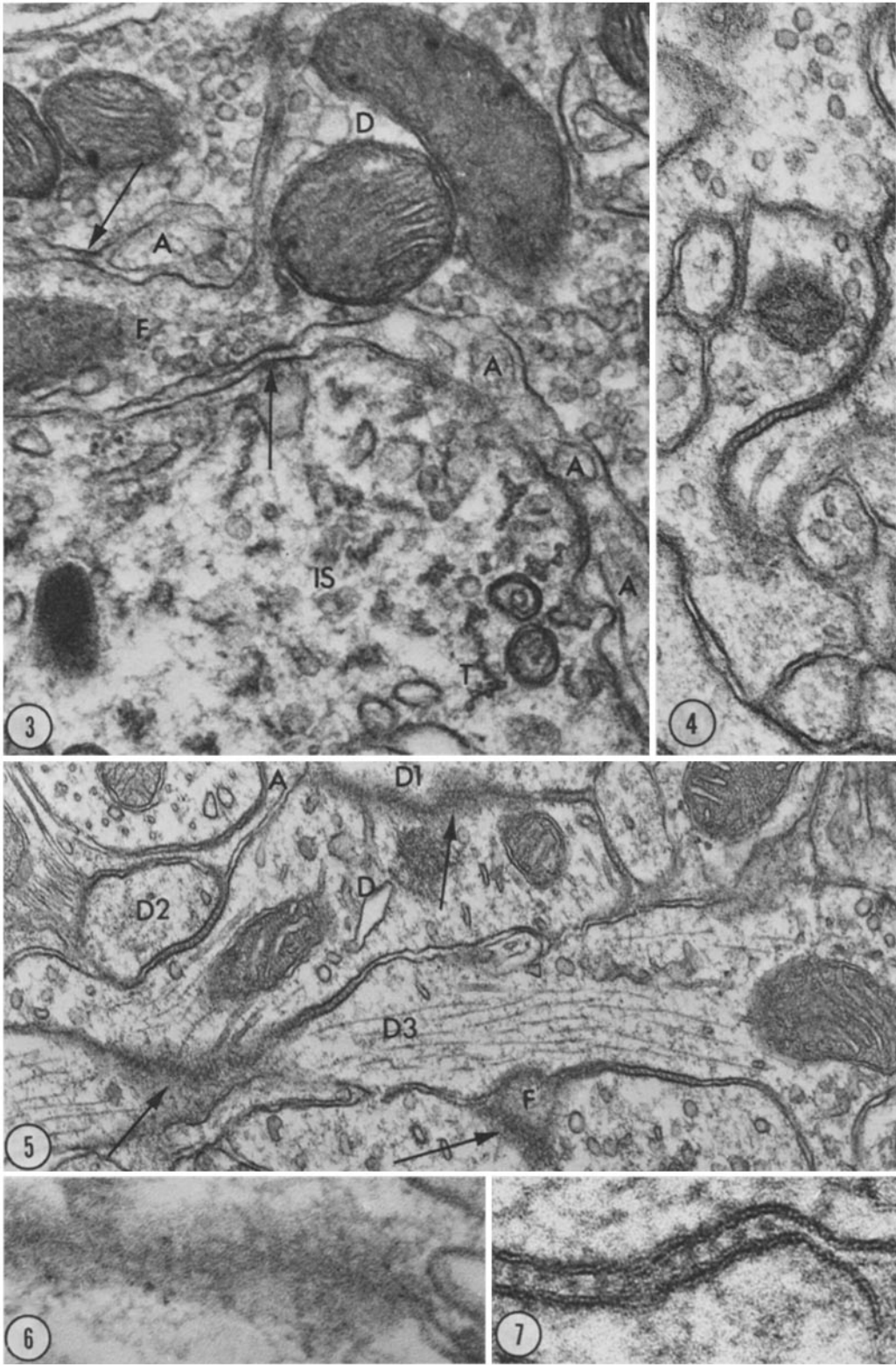
series of three or more especially between the larger distal segments, and the focal constrictions of the intercellular space at the periphery of the junctions. The septa of the axo-axonic septate junctions also differ from invertebrate septa in that the former are often inclined with respect to the cell membranes and their central portion often stains more intensely after uranyl acetate block staining.

The septate junctions of the basket formations, lacking synaptic vesicle clusters, would not appear to be directly involved in the chemical synaptic activity between the basket cell and the Purkinje cell. They are not present between the descending collaterals and the Purkinje cell body where most of the synaptic contacts are found (Hámori and Szentágothai, 1965), nor are they found where the occasional distal segment synapses on the initial segment of the Purkinje cell axon.

Septate junctions have been suggested as sites of electrical coupling in some invertebrate tissues (Loewenstein and Kanno, 1964; Satir and Gilula, 1970) and the presence of a few strategically placed sites of electrical coupling between the basket cell axon and the initial segment of the Purkinje cell (Fig. 3) would have considerable appeal in view of the profound inhibitory effect imposed on the Purkinje cell by the basket cell (Anderson et al., 1963). Since gap junctions have been identified as sites of electrical coupling (Bennett et al., 1963; Furshpan, 1964) simple extrapolations from invertebrate systems seem inappropriate at this time in view of the fact that extensive gap junctions have recently been found immediately adjacent to septate junctions in at least one invertebrate system (Hand, 1971).

In any event the above considerations fail to encompass the one enigmatic quality of the basket formation which makes it unique as a central nervous system structure, namely, that the vast majority of the distal segments do not form synaptic contacts, either with the Purkinje cell, with each other, or with any other postsynaptic structures, in spite of their abundant supply of synaptic vesicles. In fact, some descending branches proceed directly to the vicinity of the initial segment of the Purkinje cell axon without synapsing on the Purkinje cell body (Ramón y Cajal, 1909; Fox et al., 1967).

In the absence of synaptic contacts between the distal segments, the septate junctions may serve to bind small groups of these processes together, imparting some structural rigidity to the basket

formation. It would seem that distal segments without such contacts would be readily dissected apart by the omnipresent astrocytic processes. The extent of the contact relationships formed by a basket cell descending branch through its septate junctions may be a factor in determining if that descending branch establishes or maintains synaptic contact with the Purkinje cell.

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