

Subsurface Cisterns in the Vertebrate Retina*

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Summary. Structures identified as subsurface cisterns (SSC's) were found in retinal neurons and their processes in the Western grey squirrel, the California and 13-line ground squirrels, the South African clawed toad, and the domestic cat. The SSC's are located in amacrine, bipolar, and ganglion cells; they are connected with the rough endoplasmic reticulum and are associated with specific membrane specializations. SSC's were not seen in the Müller cells, an observation which agrees with earlier reports that these organelles do not exist in glial cells.

Key words: Subsurface cisterns – Retina – Endoplasmic reticulum – Neurons – Membrane specialization.

Introduction

Subsurface cisterns (SSC's) are generally considered to be distinct organelles, unique to neurons and not occurring in glial cells (Rosenbluth, 1962; Siegesmund, 1968; Weis, 1968; Pannese, 1968; Raviola and Raviola, 1969; Takahashi and Wood, 1970; LeBeux, 1972). There is, however, no known function for these unusual structures. Most authors speculate on the role of SSC's in terms of possible alteration of neuronal membrane functions, but there is no physiological or biochemical evidence for such a role. Recent findings reported by LeBeux (1972) and by us in the present paper, show that there are specific modifications of cell membranes at the site of the SSC's themselves which seem to indicate some specific role in neuronal function.

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During the course of ultrastructural studies on a variety of vertebrate retinas, we have noticed SSC's as commonly occurring in bipolar, amacrine, and ganglion cells as well as in processes in the inner plexiform layer (IPL). Our results differ somewhat from those reported by Raviola and Raviola (1969) on the rabbit's retina in terms of the locations of the cisterns as well as in the origin and structure of the organelle.

Methods

The species used in this study were the Western grey squirrel (*Sciurus griseus*), California ground squirrel (*Spermophilus beecheyi*), 13-line ground squirrel (*Spermophilus tridecemlineatus*), South African clawed toad (*Xenopus laevis*), and the domestic cat (*Felis domesticus*).

The retinas from *S. griseus* and the cat were fixed by removing the anterior portion of the eye at the *ora serrata* and immersing the posterior part for 1.5 hrs in 2.5% glutaraldehyde buffered to pH 7.4 in 0.067 M sodium cacodylate with 0.05% CaCl₂ added. The retinas of *S. beecheyi* and *S. tridecemlineatus* were fixed by intracardiac perfusion of an aldehyde-picric acid solution, modified from Ito and Karnovsky (1968) (1% paraformaldehyde, 1% glutaraldehyde, 0.02% picric acid in buffer as above). After perfusion, the eyes were immersed in additional fixative for 1.5 hrs. Following aldehyde fixation, the tissue was washed for 2 hours in buffer with 45 mg/ml sucrose added, post-fixed in 2% OsO₄ in 0.14 M veronal acetate with 0.5% CaCl₂ and 45 mg/ml sucrose added (pH 7.4). *Xenopus* retinas were fixed by immersing the posterior part of the eye in 1.3% OsO₄ in veronal acetate (as above) at 4°C for 1.5 hrs. All tissue was dehydrated in a graded ethanol-water series, transferred to propylene oxide and embedded in Araldite. Sections for electron microscopy were placed on either copper mesh or formvar coated slot grids for serial examination; stained with aqueous uranyl acetate (1.0%) and lead citrate, and examined in a Siemens 101 electron microscope.

Results

At low magnification, SSC's can be recognized as electron dense structures lying parallel and just subsurface to the plasma membrane (Fig. 1). In the inner nuclear layer of the retina, we have found SSC's in both amacrine and bipolar cell bodies (Figs. 1, 2, 4, 7, 8a), and also in ganglion cell bodies and

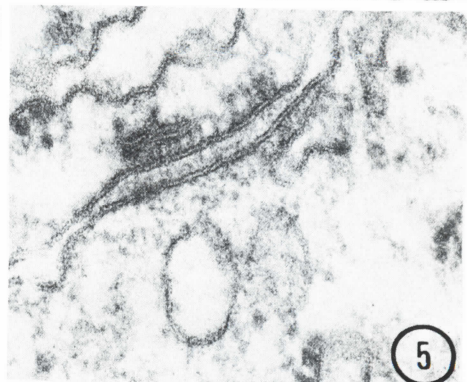
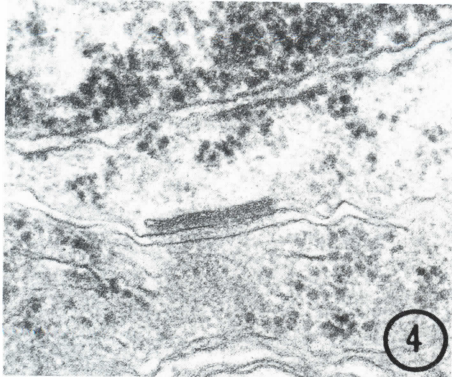
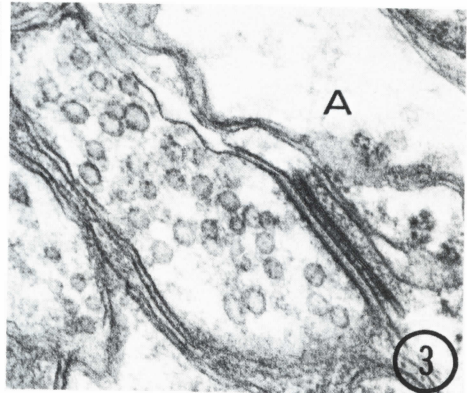
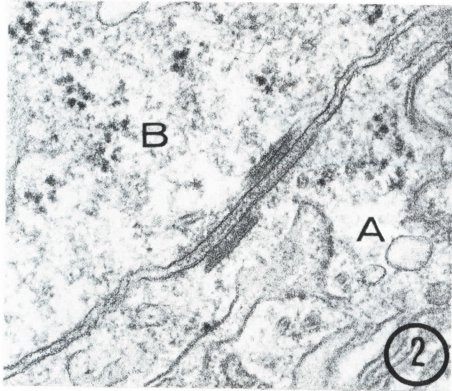
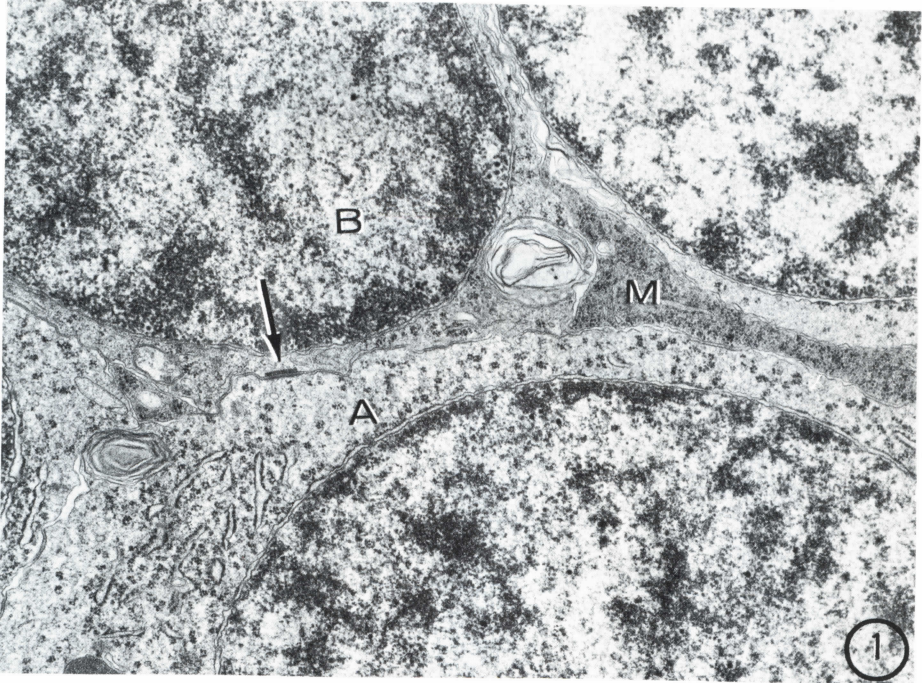
Fig. 1. Subsurface cistern (SSC) (arrow) in bipolar cell (*B*) body in retina of *S. griseus*. Amacrine cell (*A*) body opposite SSC. Also note Müller cell process (*M*). $\times 15,000$

Fig. 2. SSC's in adjacent cell bodies: amacrine cell (*A*), bipolar cell (*B*). Note widening of intercellular cleft and dense line bisecting it. *S. beecheyi*. $\times 60,000$

Fig. 3. SSC in amacrine cell body (*A*), opposite inner plexiform layer process. Note cross-bridges between SSC and plasma membrane; widening of intercellular cleft; dense material surrounding SSC; and undercoating on plasma membrane of process opposite SSC with synaptic vesicles in its cytoplasm. *S. griseus*. $\times 60,000$

Fig. 4. SSC in an amacrine cell, opposite Müller cell process. Note slight widening of intercellular cleft. *S. griseus*. $\times 60,000$

Fig. 5. Small portion of SSC showing 70 Å projections between it and plasma membrane. Both cells are amacrine. Note widening of intercellular cleft. *S. beecheyi*. $\times 150,000$



processes in the IPL (Fig. 8b). SSC's have not been found in Müller cell bodies or their processes which can be easily recognized by the electron dense staining qualities of their cytoplasm (Figs. 1, 4), nor have SSC's been seen in horizontal cells which were studied extensively in the cat and rabbit (Fisher and Boycott, 1974).

At higher magnification, the SSC's appear as flattened saccules; the structure of the individual membranes often being obscured except at the very edges of the cistern (Figs. 3, 4, 6). The membranes of the SSC are exceptionally electron dense and often appear to be associated with dense, amorphous cytoplasmic material (Figs. 3, 8b). The element opposite the SSC can be either a neuronal cell body or process, or a Müller cell body or process (Figs. 1-8). In one instance, SSC's were found in adjacent cells (Fig. 2) but were not opposite one another as reported in chick embryonic spinal ganglia neurons (Weis, 1968).

In our study, SSC's were found to be associated with specializations of the plasma membrane, including, in both species of squirrels and in *Xenopus*, a slight widening of the intercellular space (Figs. 2-6, 8b). Small (70 Å) projections were often found extending between the membranes of the SSC and the plasma membrane (Fig. 5); also, there was frequently a dense undercoating of the plasma membrane of the cell opposite the SSC, with the undercoating running the length of the SSC (Figs. 3, 8b). In the cat retina, SSC's were often adjacent to somato-somatic synapses between amacrine and bipolar cells (Fig. 7).

In the amacrine cells of squirrels and *Xenopus*, the SSC's connect to the rough endoplasmic reticulum (RER) (Figs. 6, 8a), although there seem to be differences in the morphology of the connections between the species. In *Xenopus*, the connection is obvious (Fig. 8a), while in the squirrels, the connections are made by fine, tenuous RER membranes which were only found by examinations of serial sections (Fig. 6).

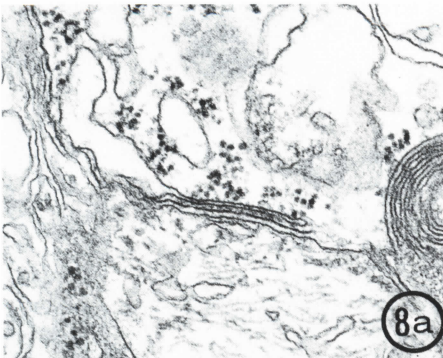
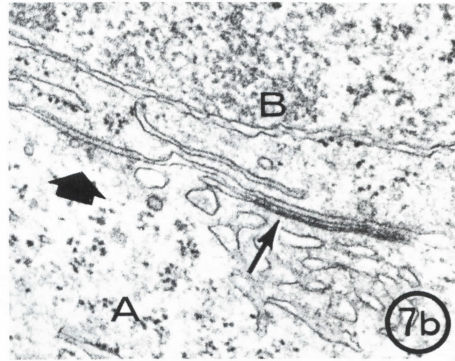
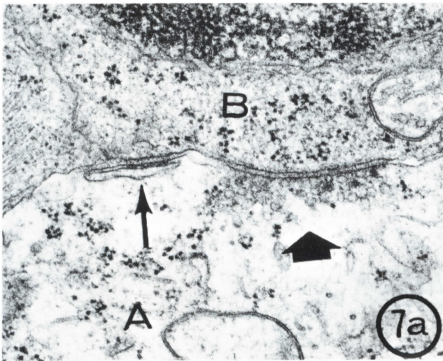
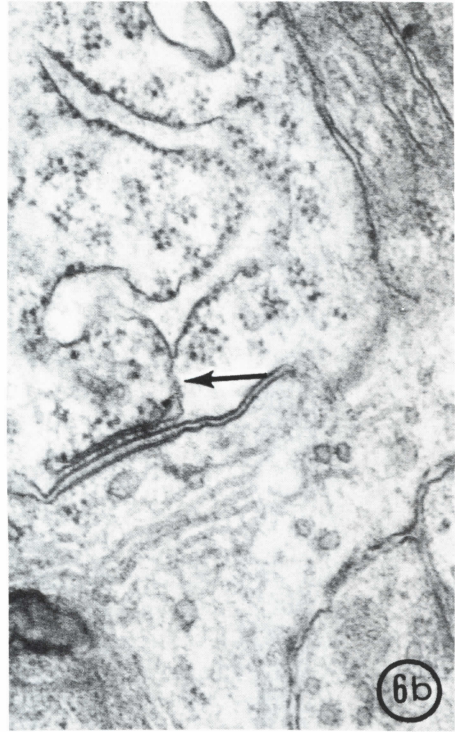
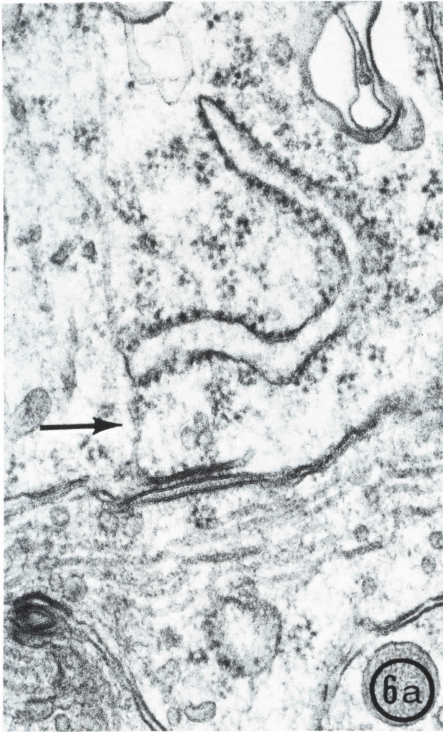
Discussion

Detailed descriptions of SSC's in the vertebrate retina have been reported in studies on the paired cones of the guppy (Berger, 1967) and on amacrine cells in the rabbit (Raviola and Raviola, 1969). This organelle has been mentioned as occurring in goldfish photoreceptors (Stell, 1967) and in primate amacrine

Fig. 6a and b. Serial sections of SSC in amacrine cell body. Note tenuous connection (arrows) between SSC and RER in both sections, separated by three sections. Note IPL process containing microtubules and small vesicles opposite SSC. $\times 60,000$

Fig. 7a and b. Two SSC's (thin arrows) in cat retina adjacent to somato-somatic synapses (thick arrows) between amacrine (A) and bipolar (B) cell bodies. In 7b the SSC is associated with irregular profiles of ER. (a) $\times 36,000$; (b) $\times 48,000$

Fig. 8a and b. SSC's in *Xenopus* retina. (a) Amacrine cell body. (b) IPL process. Both connect to RER. Note dense projections between SSC and plasma membrane and density of the membrane in the apposing cell. (a) $\times 52,000$; (b) $\times 61,000$



cells (Missotten, 1965; Dowling and Boycott, 1966). The SSC's observed in our material are similar to those described in studies of various neurons where they are considered to be a specialized part of the RER (Rosenbluth, 1962; Weis, 1968; Takahashi and Wood, 1970; LeBeux, 1972); thus, our findings differ from those of Raviola and Raviola (1969) in two major ways. We find, first, that SSC's are located in amacrine, bipolar, and ganglion cell bodies as well as in IPL processes, and second, that they connect to the RER. In the squirrel retinas, the connections to the RER might not have been found if the sections had not been examined serially because the membranes making the connection are so small and tenuous. The fact that we did not find SSC's in Müller cells is in agreement with previous reports that SSC's do not exist in glial cells (Rosenbluth, 1962; Siegesmund, 1968; Takahashi and Wood, 1970; LeBeux, 1972). Also, it seems likely that SSC's do not exist in horizontal cells, at least in the cat and rabbit, since in those species the horizontal cells were studied extensively in serial sections (Fisher and Boycott, 1974). We did not find these organelles in photoreceptors in any species examined in this study, but they have been located in developing cones of the human fetal retina (Fisher and Linberg, in prep.).

The membrane specializations associated with the SSC's as reported here are slightly different from those reported in any one past study. LeBeux (1972) described small (70 Å) projections extending between the plasma membrane and the SSC. We found similar structures linking these membranes in the retinas of the squirrels and *Xenopus*. Other reports usually describe the region between the two membranes as containing "an opaque substance" (Takahashi and Wood, 1970), a "horizontal line" (Rosenbluth, 1962), or a "fine granular material" (Siegesmund, 1968); however, these same authors mention that the two are separated by a "constant width" (50–80 Å according to Rosenbluth, 1962), which would seem to indicate that the presence or absence of the cross-bridges, described by LeBeux (1972), is a function of the fixation procedure or quality. In the present study we generally observed a slight increase in width of the extracellular space adjacent to the SSC, and a dense, amorphous substance underlying the membrane of the cell just opposite the SSC. Along the length of the SSC, the two cell membranes seem to run remarkably parallel to one another. A similar modification of the extracellular space and adjacent membrane was reported by Raviola and Raviola (1969). In certain systems, SSC's have been reported to consistently underlie synaptic contacts on the postsynaptic side (Smith and Sjöstrand, 1961; Hama, 1965, 1969; Jande, 1966; Gray and Guillery, 1966; Pappas and Waxman, 1972) and were thought to possibly play some role in synaptic transmission. In our study, SSC's were not found postsynaptically, but in some instances were seen to lie adjacent to somato-somatic synapses (Fisher, 1972) between the cat's amacrine and bipolar cells. Their significance to synaptic transmission is unknown for either location. Considering the similarity between the membrane modifications which occur at SSC's and at chemical synapses, it is tempting to speculate that they may play some role in intercellular communication, although there is, however, no physiological evidence to provide substance to such speculation. It may be significant that Brzin *et al.* (1966) localized acetylcholinesterase activity in the SSC's as well

as among other organelles in frog sympathetic and dorsal root ganglion neurons. The meaning of the occurrence of the enzyme in this location is unknown.

Unlike in previous studies, we have not observed SSC's occurring in conjunction with any organelle except the RER. Rosenbluth (1962) and Takahashi and Wood (1970) reported a special relationship between mitochondria and SSC's, while Copeland (1966) found them frequently next to desmosomes. Raviola and Raviola (1969) saw stacks of SSC-like membranes associated with lamellae of the smooth endoplasmic reticulum deep in the amacrine cell cytoplasm. They suggested that a migration of these deep-lying structures may occur to form stacks of SSC's adjacent to the plasma membrane. LeBeux (1972) described similar structures in cat cerebral neurons, but did not conclude that they undergo such a migration; rather he considered both them and the SSC's to be specializations of the RER. We observed similar lamellae only in the amacrine cells of the 13-line ground squirrel and they were not associated with SSC's. Furthermore, no stacked SSC's were found in any of our material.

From our results it seems likely that SSC's occur in the cell bodies and processes of amacrine, bipolar, and ganglion cells in the vertebrate retina. It also seems likely to us that all SSC's originate from the RER and retain a connection with it, although the connection itself may not be evident in any single thin section. Our results agree with those of most other authors, in that the cell opposite the SSC can be neuronal or glial, but that the SSC's only occur within neurons. The exclusion of these organelles from glia and the high degree of specialization of the adjacent cytoplasm and membranes seem to indicate that SSC's are involved in neuronal function, although their specific role remains unknown.

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