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***Research Article** (Open Access)*

MORPHOHISTOLOGICAL ASPECTS OF THE SACCUS VASCULOSUS IN *LIZA PARSIA* (HAM.) FROM THE COASTAL AREAS OF BAY OF BENGAL

***Sanjib Bose[1]**
[1]*Department of Zoology, Sreegopal Banerjee College, Bagati, Mogra, Hooghly-712148, W.B., India*
** Author for Correspondence*: *sanjibbose007@gmail.com*

ABSTRACT

The histoarchitecture of different cells in the epithelial lining of the saccus vasculosus (SV) and their role in the physiological functions in brackish-water teleost, *Liza parsia* (Hamilton, 1822) from the rivers of the Gangetic delta were investigated by using various staining techniques. The sac like saccus vasculosus located at the ventral surface of the diencephalon close to the hypophysis but clearly separated from it. The epithelium of the saccus vasculosus is composed of large number of coronet cells and small number of supporting cells. The organ consists of single layer of stratified epithelium and intricate system of channels. The lumen of the organ is rich in secretory substances from the coronet cells which in turn drain it into the third ventricle of the brain for mix with cerebro spinal fluid. The apical parts of the coronet cell exhibit various shapes of globular protrusions with definitive stalk. The terminal ends of these cells are attached with the blood vessels and nerve fibers. This is supposedly the first attempt to study saccus vasculosus of a brackish-water teleost from its wild habitat.

***Keywords*:** *Histoarchitecture, Histophysiology, Saccus vasculosus, Brackish-water, Liza parsia*

INTRODUCTION

The brackish water teleost *Liza parsia* is widely distributed in the gangetic delta estuaries of Bangladesh, India (including Andaman Island), Pakistan and Sri Lanka (Talwar and Jhingran, 1991). It was less studied compared to other teleosts because of its less availability in the reverine confluences owing to its marine habitation and spawning habit. The saccus vasculosus (SV) is embryologically an ependymal and circumventricular organ of the hypothalamus of several elasmobranches and teleosts. This organ protrudes from the diencephalic caudal infundibulum, juxtaposing the pituitary gland in fishes (Dorn, 1955). The third ventricle of the brain is in continuum with the lumen of this organ that consists of epithelial layer or meninx which contains sinus-like blood vessels and ordinary capillaries (Mellinger 1960). It is connected with the brain by the bundles of axons of the neuronal cells and serves as connective link between cerebro spinal fluid (CSF) and blood vascular system (Watanabe, 1966). The neuroepithelium layer of the organ is composed of highly specialized coronet or crown cells and supporting glial cells with fluid like substances containing neurons (Joy et al, 1979; Sueiro et al, 2007). On functional aspect this organ is chiefly dealing with secretion of various mucopolysaccharides via coronet cells and act as a storage site to the brain. Other functions include chemoreception, ionic regulation, pressure reception, and the glucose loading of the CSF by means of transporting low molecular weight substances into and from this fluid (Marquet et al, 2005; Yne et al, 1997). This organ is large in sea-fish, smaller in river-fish, and almost lacking in fishes of canals and ponds, which suggests its function is to estimate the pressure of the water, and therefore the depth at which the fish is swimming (Dammerman, 1910). The present study attempts to assess the histological architecture of various cells lining the epithelium of SV of *Liza parsia* to understand possible physiological role of the cells concerned and because it is probably the first attempt to study the organ in a brackish-water teleost, it may fill some of the lacunae still exists.

MATERIALS AND METHODS

Live adult mature fishes of *L. parsia* (18-24 cm in total length) were collected from West Bengal Govt. brackish water fish farm in Junput, District- Purba Medinipur, West Bengal, India and brackish-water rivers of Sunderban, W.B., India. Fishes were sacrificed by decapitation following the guidelines given by the Institutional Ethical Committee. The brain mass including the saccus vasculosus was exposed from the ventral side of the head and was initially fixed *in situ* with 10% neutral formalin. After few minutes the SV along with the rest of the brain were carefully detached from the cranium and was fixed in aqueous Bouin's fluid for 16-18 hour. After fixation the tissues were washed thoroughly several times in 70% alcohol, dehydrated properly through an ascending series of ethyl alcohol and cleared in benzene. Then the tissues were infiltrated in paraffin wax of 56-58˚C under a thermostat vacuum paraffin embedding bath for 1 hour and paraffin blocks were prepared. The serial sections were cut at 4µ thickness using a rotary microtome (Weswox). The dewaxed sections were stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple stain (MT) (Mallory, 1936) and PAS-orange G (PAS-OG) stains.

RESULTS

The saccus vasculosus in *L. parsia* is a sac like structure, moderate in size, deep red in colour due to the presence of numerous blood vessels and enclosed by loose connective tissue also known as the duramater (Figs. 1-4). It is situated on the ventral surface of the diencephalon near the third ventricle, postero-dorsal to the pituitary gland and has no clear connection with it. There are numerous loculi of various shapes and sizes and these are surrounded by sinusoids of blood inside the organ (Figs. 1-5).

The SV in *L. parsia* consists of follicular structure lined with different levels of cell nuclei. It is devoid of a common central cavity and it is more or less composed of lumens of simple or branched loculi with intercommunicating channels (Figs. 1-5). Few large vesicles are visible in the ventral portion of SV, which are irregular in shape and surrounded by vascular element (Fig. 6). The capsular wall of the saccus, invaginates the parenchyma to form villi like projections towards the lumen in few places. These villi is made up of outer stratified epithelium, lined by monolayer of coloumnar or cuboidal cells whereas, the blood vessels forms the core (Figs. 4-8).

The predominant cuboidal or coloumnar cells with distinct basement membrane are known as coronet cells, which are specialized cells of saccus epithelium. The large ovoid nuclei of the coronet cells are located at the center or basal margin. These cells are 5-8µ to 16-32µ of measured size (Figs. 6-10). There are some elongated or triangular sand glass-like supporting cells comparatively smaller in size with centrally located large nuclei, scattered in between the coronet cells with slightly less dense cytoplasm and are attached with basal lamina. Prominent nuclei in few supporting cells were found. The apical portion of some coronet cells with characteristic apical protrusion, are continuous with the abundant quantity of basophilic secretory luminal materials (Figs. 6-10).

The tinctorial properties of the cytoplasm of the coronet and supporting cells are more or less similar although supporting cells shows higher density of cytoplasm than that of the coronet cells. Moreover coronet cells are having apical globular protrusions which are projecting towards the lumen of the SV while supporting cells are devoid of any apical protrusions as such (Figs. 6-10). In the present observations the saccus epithelium in *L. parsia* is contacted with nerve terminals which in turn connected through the axons from the brain to the third ventricle (Figs. 3, 4, 6, 7, 9).

Sometimes in the saccus epithelium, some large extracellular spaces were visualized which are surrounded by supporting cells or glial cells. These spaces are devoid of any blood cells and unlike of typical endothelium or blood capillaries (Figs. 4, 6). The lumen of the SV in *L. parsia* is large and abundant with basophilic secretory granules.

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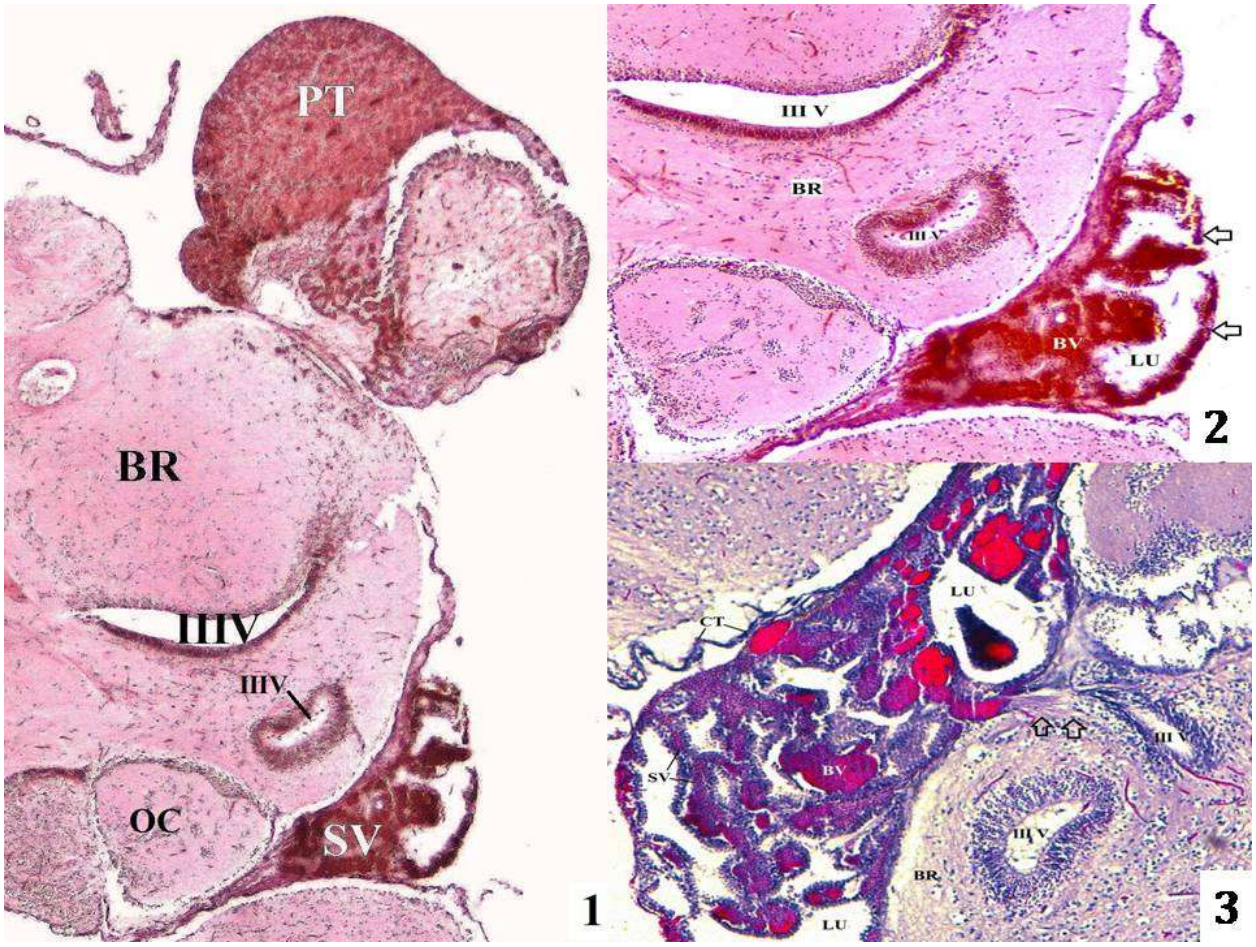

Fig. 1- Sac like saccus vasculosus (SV) attached with brain (BR) situated beyond the pituitary (PT) posteriorly. Note the location of the optic chiasma (OC) and third ventricle of the brain (IIIV) close to saccus vasculosus. (PAS-OG) × 50 X.

Fig. 2- Showing the location of saccus vasculosus (SV) enclosed by connective tissue (arrow) on the ventral side of the brain (BR) and close to the third ventricle of the brain (IIIV). Note the presence of spacious lumen (LU) and blood vessels (BV). (PAS-OG) × 100 X.

Fig. 3- Photomicrograph showing almost triangular shape of SV invaded by Blood Vessels (BV). The lumen (LU) of SV provided with villi like projections. Note the presence of axonal fibers (arrow) connecting the blood vessels from the SV to the third ventricle (IIIV) of the brain (BR). (MT) × 100X.

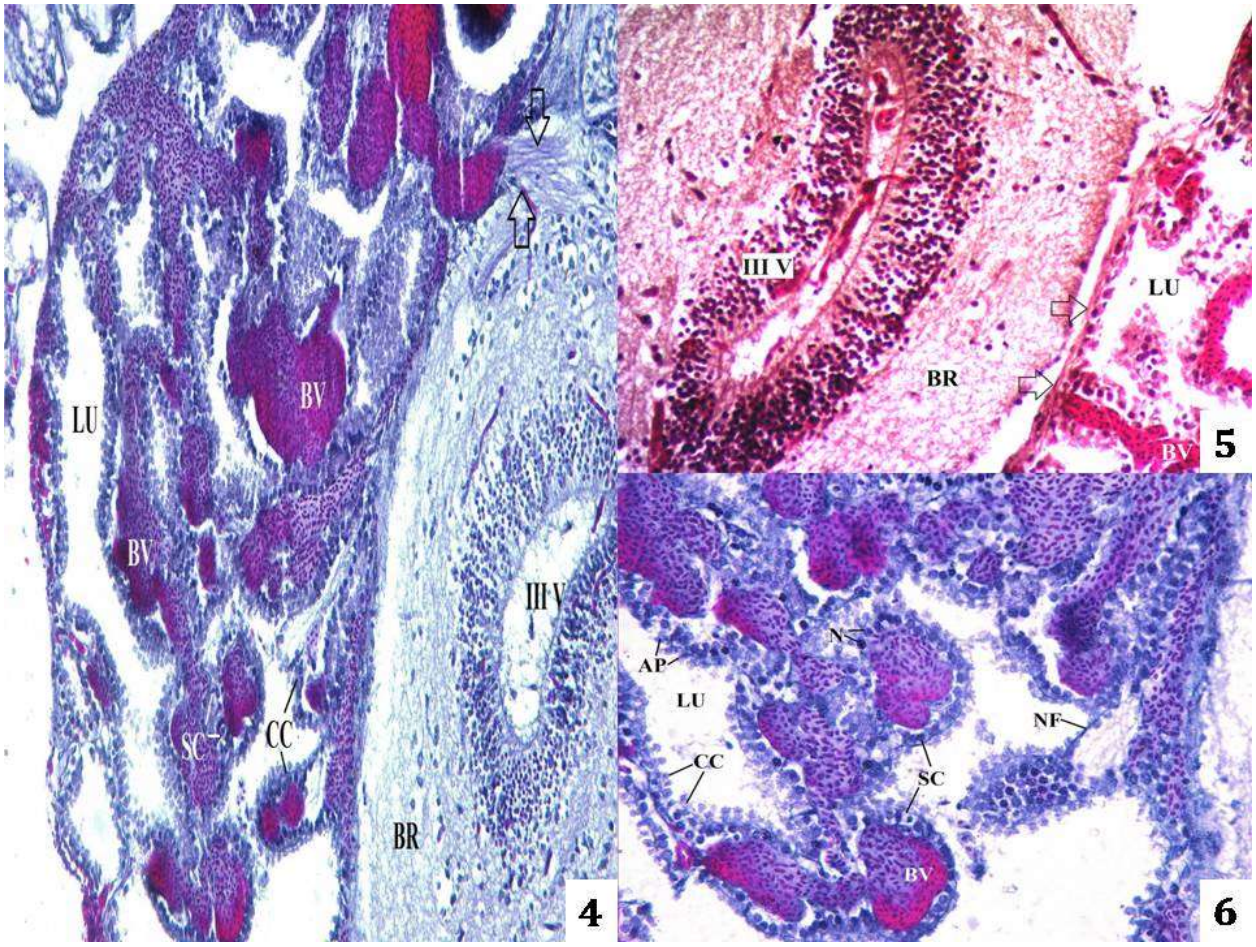

Fig. 4- Showing coronet cells (CC) scattered with supporting cells (SC) close to the blood vessels (BV) in the saccus epithelium. Note the presence of nerve fibres (arrows) and blood vessels (BV) proximal to the third ventricle (IIIV) of the brain (BR). The lumen (LU) of the SV has villi like projections from the epithelium. (MT) × 200 X.

Fig. 5- Photomicrograph showing separation of SV from the brain (BR). Note the lumen (LU) of SV which are richly supplied with blood vessels (BV). Note the presence of duramater (arrows) which covers the SV and proximity of the third ventricle of brain (IIIV). (HE) × 200.

Fig. 6- Showing the arrangements of coronet cells (CC) within the loculi of the saccus vasculosus which are surrounded by blood vessels (BV). Note the prominent nuclei (N) of CC. Note also the presence of supporting cells (SC) in between CC and nerve fibers (NF) with the neuronal contact with CC. Apical protrusions (AP) of the CC are also visible in the lumen (LU). (MT) × 400.

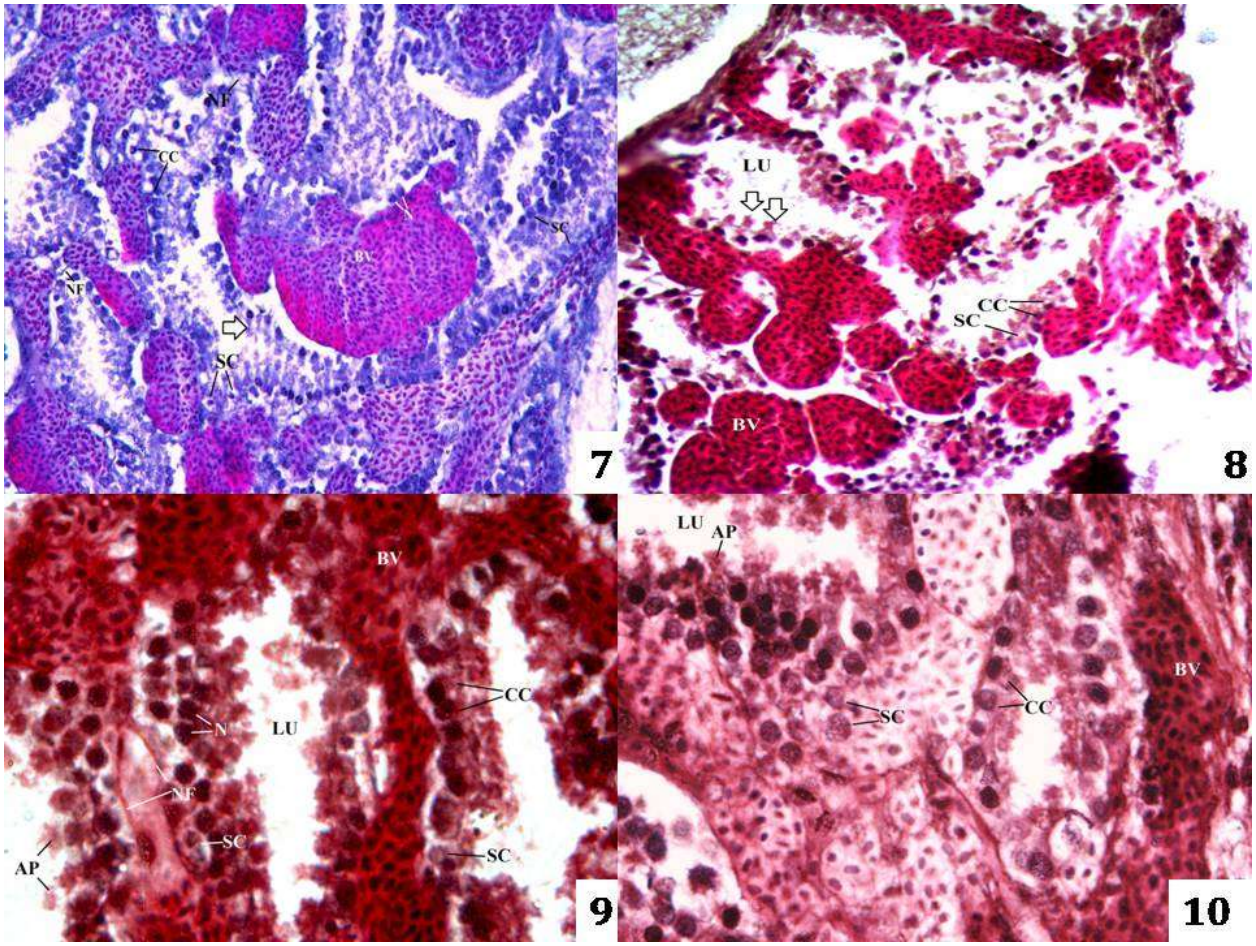

Fig. 7- Photomicrograph of the saccus vasculosus of *L. parsia* showing the nerve fibers (NF) penetrate into the epithelium. Coronet cells (CC) with prominent nucleus (N) and intermingling supporting cells are also visible. Note apical protrusions and secretions (arrow) from the CC. The interstitial tissue intervening the follicles contains a number of blood capillaries (BV). (MT) × 400.

Fig. 8- Arrangement of CC on the free border of villi like projection of SV. Note presence of supporting cells (SC) in between CC and BV behind the series of CC with secretory material. Apical protrusions from the CC are also visible in the lumen (LU). (HE) × 400.

Fig. 9- The arrangement of coronet cells (CC) of *L. parsia* with prominent nuclei (N) in higher magnification within the saccus loculi connected with blood vessels (BV). Note apical secretion (AP) of CC, supporting cells (SC) with less chromatic nuclei and nerve fibers (NF) from the CC. (HE) × 1000.

Fig. 10- Photomicrograph showing CC and SC in higher magnification with underlying BV. Note the deeply stained nuclei of CC and stainable secretory materials and apical protrusions (AP) in the lumen (LU). (HE) × 1000 X.

DISCUSSION

The saccus vasulosus (SV) is an essential organ and found in most primary freshwater teleosts. Although, some secondary freshwater teleosts and some marine teleosts swimming in the surface water possess a reduced SV, but other secondary freshwater and marine teleosts have a well-developed SV (Tsuneki, 1992). Saccus vasculosus is generally situated posterior to the pituitary gland though in some teleosts it is reported to lie opposed to the hypophysis (Prasada Rao 1966). The SV has an attachment with the floor of the diencephalon as saccular protrusion of the caudal infundibular wall of the diencephalon in most of the fishes causing its proximity towards the hypophyseal complex. In the present investigation, the brackish-water teleost *L. parsia* possess a moderate sized oval sac like SV, located ventral orifice of the diencephalon near the third ventricle, postero-dorsal to the pituitary gland and has no clear connection with it. It is light red in colour due the presence of numerous blood vessels inside the organ. Most of the Osteichthyes and Chondrichthyes possess infoldings of the epithelium of the SV wall into the cavity which opens into the third ventricle of the brain and the epithelium having CSF and the endomeningeal fluid at the opposite sides. The epithelium surface enlargement between two fluid compartments facilitate the exchange and trans-epithelial transport, absorption and secretion process and may also be responsible to provide nutritive substances (Herring, 1908; Dammerman, 1910; Jansen *et al.,* 1969). There are rich vascular supplies inside the SV and this was attributed as a gland of brain and designated a secretory role of unknown function (Kamer, 1977). In the posterior region of the infundibulum, haemopoietic tissue and blood vessels are abundant and this may be due to secretory activities of the SV in some fishes (Prasada Rao, 1966). Kamer (1977) also reported rich vascular supply in SV and interpreted SV as a gland of brain with similar function. Nakane *et al.,* (2013) reported the SV serves as seasonal sensor that has the capacity to respond to photoperiodic signals in fishes.

In the present study similar infoldings or villi in the epithelium linings of the SV in *L. parsia* was observed and the probable cause of this may be in line with the previous findings. Moreover this villi like epithelium is composed of the coloumnar coronet cells and the supporting cells but they are not distributed alternately as reported in *Mystus vittatus*, *Callichorus pabda* and *Bagarius bagarius* (Singh and Sathyanesan, 1964). The ependymal coronet cells are specialized cells having apical cytoplasmic outgrowth, called globules. In *Ompok bimaculatus* apical globules are lacking, but apical region shows signs of secretory activities (Ghosh and Chakrabarti, 2013). The supporting cells or tanycytes (Jansen et al, 1981) are smaller cells and situated in between the coronet cells as heterogeneous cell population. Rossi and Palmbi (1976) have investigated The coronet cells structure in the SV of marine larval stage of *Anguilla anguilla* the smaller lumen of the SV and the vesicles present inside the apical globules of the coronet cells are found with rich electron dense material in the marine larval stage, while the same are lacking in the freshwater adult forms with expanded lumen. The cytoplasmic content of the coronet cells in the SV of rainbow trout, are composed of SER composed of connecting tubules near the basal region (Shimada, 1970). In present investigation, the supporting cells are located near the basal region of the coronet cells and the apical protrusions in the coronet cells with the secretory materials are evident in the observation. In *L. parsia* coronet cells are connected with nerve terminals that help SV to serve as a chemoreceptor organ and also possibly help to maintain composition of the cerebrospinal fluid. Similarly cerebrospinal fluid containing neurons are observed in the saccus epithelium in rainbow trout (Corujo *et al.,* 2005).

CONCLUSION

The SV of *L. parsia* composed of a network of blood capillaries and sinusoids. The globular protrusions from the tip of the coronet cells are loaded with secretary materials which is further unloaded into the lumen of the SV. These secretory materials are transported to the third ventricle of the brain via large collecting channels.

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