Routes of Nasal Olfactory Mucosal Transport of Therapeutic Agents, Microorganisms and Amoebae to the CNS Bypassing the Blood Brain Barrier

(Drugs to treat Alzheimer's, Parkinson's and such delivered using olfactory mucosal route)

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As therapeutic agents are evolved to treat central nervous system (CNS) afflictions, the blood brain barrier (BBB) has prevented the use of many of these agents for treating neurodegenerative diseases such as Alzheimer's, Parkinson's, and other CNS diseases. The blood brain barrier (BBB) blocks the use of many of the newer engineered-discovered drugs that can protect neurons, promote nerve repair and treat CNS diseases. This problem is partly resolved by the use of intranasal olfactory mucosa to deliver the therapeutic agents to the central nervous system bypassing through the BBB. [1-9, 13-20, 25] Such a method provides a simple, realistic, and rapid route of delivery into the CNS, bypassing through the BBB, because of the unique connections and transportation routes between the nose olfactory mucosa, olfactory nerves, olfactory bulb, subarachnoid space cerebro spinal fluid (CSF) and CNS (figures 5-7) than any other histological and anatomical structure and site. [1-9, 10, 11, 21, 22, 29] We want to explore and explain how these therapeutic or non-therapeutic agents such as brain eating amoeba, meningococcus and rabies virus and such reach the brain, bypassing through the formidable BBB.[12, 24, 26] We have studied the membranes of the CNS since 1960 including olfactory mucosa, and want to discuss in the light of delivering therapeutic agents to CNS.[13-16]

Various formulations of therapeutic, pharmaceutical, biochemical, and biological agents or compounds have been successfully delivered to the CNS using intranasal olfactory mucosal delivery in a variety of species already. We have used insulin (unpublished, patented) to treat and improve memory in every neurodegenerative disease, including Alzheimer's and Parkinson's disease, as well as in healthy students to improve memory, for more than two decades. The results are amazing improvements from the disease state, and improved memory as also reported by Craft, Frey III and others [1, 11, 12, 21, 22, 25] indicating paucity of insulin in the CNS to supply much needed glucose to neurons. That is why de la Monte aptly called Alzheimer's disease as "diabetes type III of the brain". [10, 11] The insulin thus delivered through olfactory mucosa binds to the insulin receptors found on the neurons of CNS, resulting in rapid and additional glucose uptake needed by the neurons for proper functioning. [9, 20]

Delivery of protein therapeutic agents to the CNS clearly involves extra-neuronal transport as it occurs within 30 minutes rather than hours (e.g. cephalexin).[5] Our histological findings support that it passes between intercellular spaces between receptor and supporting cells of the olfactory mucosa, (figures 1-4) subsequently reaching the sub perineural epithelial and inter-axonal spaces in the 20 short olfactory nerves with CSF, (figures 4-7) afterwards it is transported to the CSF around the olfactory bulb and reaches the brain and spinal cord by CSF circulation (figure 7). A number of diverse therapeutic agents microbes and ameba have been found in CNS tissues following intranasal olfactory mucosal administration.[1-8, 12, 22, 24, 25, 26, 30] Many hormones, addictive drugs such as cocaine, narcotics, naloxone, and such agents easily reach the CNS rapidly, bypassing through the BBB though the route we describe thereafter, not through the trigeminal nerve branches as hypothesized. [1, 22, 25] (figures 8, 9) The studies show that the delivery to the brain from the olfactory mucosa decreases with increasing molecular weight of the drug, this limits what we can deliver and treat. We studied the olfactory mucosa of the squirrel monkey and other species of experimental animals with brief reference to the work of our and others' study of the olfactory mucosa. [13-16, 23, 29-31] Our intent was to show, these therapeutic agents and microorganisms take to reach the CNS, bypassing the BBB. Our studies and other studies are incorporated here even though it is impossible to cite all of them.[1-29] Recently, Dahlin et.al.[30] successfully visualized the olfactory epithelial drug distribution and transferring pathway from the olfactory mucosa to the CNS using the model drug 3kD fluorescein dextran (FD3).

This article provides an overview of the unique anatomic, histologic, and physiologic characteristics of the nasal olfactory mucosa and its connections that allow certain intranasally applied drugs and microbes

transported across the nasal respiratory mucosa and olfactory mucosa, and subsequently be transported directly into the CNS.

Anatomical and histological aspects of olfactory mucosa as transportation route to brain by passing BBB

The olfactory mucosa is situated within the recesses of the skull, under the cribriform plate of the ethmoid bone that forms the roof of the nose, situated 7 cm from the nostril, being positioned partly on the nasal septum and partly on the superior turbinate (Figure 1).[2-8] Olfactory mucosa is not easily accessible in humans because it is located in 1.5 mm crevices between closely apposed superior turbinate of the lateral wall and nasal septum. It is important to note that the therapeutic agents need to be delivered to this narrow passage to treat CNS afflictions as described below (figure 10). The olfactory mucosa is made up of a mucus layer situated on the top of the receptors cells, supporting cells between the receptor cell, basal cells below the receptor and supporting cells, and goblets cells extending from lamina propria opening on the olfactory mucosa supplying the mucosal coating to the olfactory mucosa (figure 2). The lamina propria, below the basal cells, has 20 olfactory nerve bundles with BV (ethmoidal) and lymphatics (deep cervical) surrounded by connective tissue, which form the epi and perineural connective tissue around the olfactory nerves.

There are 10-23 cilia from each receptor cell (extension of receptor cells dendrite) and microvilli of the sustenticular cells embedded in the mucosal layer. They are entangled in a thick viscous layer of mucus from the goblet cells secretion from the lamina propria that does not allow them to move and also may not participate in the transport of olfactory mucosa delivered therapeutic agents. Based on our studies, there is a possibility that CSF seeps from the olfactory nerves between these cells and emerging axons supplying the neurotrophic factors, nutrition, and at the same time keeping olfactory mucosa wet, (figure 5).

Typically, ± 20 axons are bundled and surrounded by Schwann cells. Collection of these bundles form nerve fasciculi of olfactory nerves (20 olfactory nerves trunks or fasciculi) surrounded by perineural epithelial cells, [13-16] **not by Schwann cells** [2, 27, 28], creating sub perineural epithelial and inter-axonal spaces around each nerve fasciculus. In many cases, the perineural epithelial cells are mistakenly identified as Schwann cells.[2, 27, 28] Our study for decades has shown that they are an extension of pia-arachnoid mater extension from the olfactory bulb leptomeninges, and the leptomeninges also covers the entire peripheral nervous system from the rest of the CNS (including sensory and motor end organs) akin to brain meningeal pia-arachnoid (leptomeninges) coverings, not derived from Schwann cells. [13-16]

Route of transport of various therapeutic, pharmaceutical, biochemical, and biological agents or compounds from the olfactory mucosa

There are two routes taken by the various therapeutic, pharmaceutical, biochemical, and biological agents or compounds and microorganisms delivered to the surface of the olfactory mucosa. They are:

a) The first phase is the internalization of the therapeutic agents into the primary neurons of the olfactory epithelium-transcellular (by receptor endocytosis via receptor mediated uptake or passive diffusion through dendritic projections) and transport by intracellular axoplasm to the olfactory bulb with subsequent possible distribution to entorhinal cortex and other cortical areas (figure 7). Drugs from the olfactory mucosa are transported intracellularly in the olfactory neurons (axoplasmic axon transport) and supporting cells. The therapeutic agents' supporting cell has no way to empty, except through the base to the inter axonal area and transport to adjoining basal cells by passive diffusion. Therapeutic agents enter the olfactory receptor neurons by methods of endocytosis or pinocytosis. From the dendritic knob, they travel along the axon, that exits between the basal cells, and come out as axons loaded with therapeutic agents. As they traverse through the receptor cell axoplasm, they may receive some of the therapeutic agents from the supporting cells by passive diffusion.

These axons emerging between the basal cells group into 20 olfactory nerve trunks formed by the olfactory nerve fasciculi in the lamina propria. From here, the olfactory nerves cross the cribriform plate within and reach the olfactory bulb and olfactory glomeruli as first order of synapse with tufted – mitral cells. The presence of axonal pathway has been described by several authors for transport of different materials from the olfactory region to the more distant brain tissues of the CNS such as gold particles, aluminum lactate, and wheat germ agglutinin-horseradish peroxidase [2, 29] as well as viruses.[26]

This axonal olfactory nerve pathway involves uptake of the agents into the olfactory receptor neurons and subsequent axoplasmic transport of viruses, dyes, some inorganic metals, and other large molecular weight (MW) substances have been shown to enter the olfactory bulbs via this route after entering the olfactory nerve cells. This pathway was characterized by extremely slow transport, which usually takes about 24 hrs. to days to reach the olfactory bulb and deep centers in the brain (figure 7).

b) The second phase is the olfactory epithelial pathway that involves epithelial transferring by passive diffusion across the epithelium or paracellularly through the junction of the supporting cells or other means. It is significant for low MW drugs (< 1000). Compared with the slow transporting rate of the olfactory nerve pathway, the paracellular or transcellular absorptions are considered to be quick and possibly account for most of the rapid appearances of several drug molecules in the CSF and brain, such as dihydroergotamine,[6] cocaine,[3] lidocaine,[5] and cephalexin.[18] Recently, Jansson and Bjork[19] successfully visualized the olfactory epithelial drug distribution and transferring pathway into the OB using the model drug 3kD fluorescein dextran (FD3). Thirdly, Thorne [17, 20] and Frey [10] presented preliminary evidence that the trigeminal neural pathway may also be involved in rapidly delivering some protein therapeutic agents to the brain following intranasal administration. Our studies show very little if any therapeutic agents spread by this route from drug delivered to olfactory mucosa.
The second route of absorption of the drug from the submucosa, between the olfactory receptor cells and the olfactory sustentacular epithelial cells, either by para-cellular or inter-cellular mechanisms followed by uptake into the CSF around the olfactory bulb then to the CSF cisterns, especially suprachiasmatic and interpeduncular cistern of the subarachnoid space of the CNS. This method of transport is very fast compared to the trans-axonal pathway and is the main route of transport of therapeutic agents from olfactory mucosa bypassing BBB. The therapeutic agents appear very fast, with drugs appearing in the CSF in the brain and spinal cord in less than 30 minutes.[5] This is a direct spread along the anatomical routes between the sustentacular cells and the olfactory receptor neurons into the CSF, which relies on a direct anatomic connection between the submucosa and the subarachnoid extensions of the perineural space surrounding the olfactory nerves, and inter-axonal spaces as they penetrate the cribriform plate (Figure 6) communicating with the subarachnoid space of the olfactory bulb - an extracellular route.

The therapeutic agents from olfactory mucosa enter the sub perineural epithelial inter-axonal spaces surrounding the axon as they emerge from the basal cell layer. The axons are surrounded by Schwann cells in groups of bundles as shown in the electron microscopic pictures (figure 4). The group of them form nerve fasciculi and are surrounded by perineural epithelial cells [16] **not by Schwann cells as reported** [27, 28], which form sub perineural epithelial and inter-axonal spaces (figures 2-5, 8, 9) in the individual olfactory as well as in peripheral nerves, thus forming 20 olfactory nerves trunks in humans. Through these spaces, the CSF around the olfactory bulb communicate (figure 5), in the same manner by acting as a water way to relay therapeutic agents to the subarachnoid space around the olfactory bulb, then on to the subarachnoid space around the brain and vice versa.

In our dissection and histological studies, we found that some of the axons, as they are bundled to form olfactory nerves below the basal cell, are not covered with perineural epithelial cells as shown in figure 2B.[16] The colloidal gold and IFG-1 delivered to olfactory mucosa showed that the gold particles and IGF-1 were found in the endothelial cells of blood vessels in the lamina propria, in the lamina propria and in the lymphatics.[4, 29] Such particles originate from the base between the basal cell as the axons emerge. The colloidal gold IGF-1 was transported below the basal cell, leaked to the lamina propria, due to lack of perineural epithelial covering, spread to lamina propria and picked up by the BV and as well as in the lymphatics. Our finding also supports that the enlargement of deep cervical lymph nodes is also due to leaking of the CSF in between the axons of the olfactory nerves foreign material at this anatomical site, picked up by the cervical lymph nodes. As these nerve bundles, not covered by perineural epithelial cells, as they traverse in the lamina propria, gradually get the perineural epithelial cell covering like other olfactory nerves as shown in the figure 2A and B. [16]

It has been shown in a rat model that large molecular weight drugs, such as protein nerve growth factor (MW 37 kDa), insulin (MW 6 kDa) and vasoactive intestinal peptide (VIP) (MW 3.5 kDa) can be transported rapidly into the CSF. Our own study in humans shows that the insulin is rapidly transported to the CNS, bypassing the BBB. These therapeutic agents and select microorganisms are able to use the transportation route created by perineural epithelial covering of olfactory nerves creating sub perineural epithelial and inter-axonal spaces.[24] It is significant for low molecular weight drugs (MW < 1000) compared with the slow intracellular trans-axoplasmic transporting rate of the olfactory nerve pathway. The para cellular or intercellular absorptions through the sub perineural epithelial and inter-axonal spaces is rapid and possibly accounts for most of the rapid appearances of several drug molecules in the spinal CSF and the brain, such as dihydroergotamine, cocaine, lidocaine, and cephalexin. Recently, Dahlin et.al., (2000) [32] successfully visualized the olfactory epithelial drug distribution and transferring pathway into the olfactory bulb using the model drug, 3kD fluorescein dextran (FD3). This evidence is due to the spread through the sub perineural epithelial spaces around the olfactory nerves connected to the subarachnoid space around the olfactory bulb and central CSF subarachnoid space cisterns. As explained, the anterior ethmoidal branch of the trigeminal nerve and small branches of sphenopalatine ganglion are not involved in the rapid transfer of therapeutic agents as proposed, though they may participate in the slow spread to CNS CSF.

Mode of spread and final destination of therapeutic agents from the olfactory mucosa

Here are the routes taken by the therapeutic agents from the olfactory mucosa through intra-neuronal and extra neural pathways to various centers of the CNS:

1. Intra-axonal (transcellular-axoplasmic) spread results when the therapeutic agents deposited in the olfactory mucosa, endocytosed by receptor cells dendrites, then to axons, olfactory nerves, olfactory bulb, glomeruli, through the olfactory tracts axons, to mitral and tufted cells, then to olfactory tubercle, amygdala, the prepyriform cortex, the anterior olfactory, nucleus and the entorhinal cortex as well as to the hippocampus, hypothalamus and thalamus (figure 7). This is a very slow spread.
2. Spread from the intercellular and para-cellular spread between receptor and supporting cells, therapeutic agents reach the lamina propria due to paucity of perineural epithelial cell covering around the emerging axon bundles, (figure 2B) then to BV and deep cervical lymph nodes.
3. Majority of the therapeutic agents and microorganism are transported between the supporting cells, receptor cells, and dyeing receptor cells in the olfactory mucosa. At any given time about 10% of the receptor cells are dying creating a space for transport of therapeutic agents and microorganisms to the sub perineural epithelial space around the 20 olfactory nerves (figures 2-6).

4. From sub perineural epithelial and inter-axonal spaces of the olfactory nerves, the therapeutic agents spread around the olfactory bulb subarachnoid space CSF (figures, 1, 5, 8, 9), olfactory nerves passing through the cribriform plate of the ethmoid bone.
5. From olfactory bulb subarachnoid space CSF, the therapeutic agents are transported to the CSF in the subarachnoid space, specifically to the suprachiasmatic and interpeduncular CSF cisterns (figure 5),
6. From these subarachnoid space CSF cisterns, the therapeutic agents spread to the temporal lobe, hypothalamus, thalamus, amygdala, entorhinal cortex, hippocampus, prefrontal cortex and such. That is why we believe that the therapeutic agents to treat Parkinson's, Alzheimer's and other neurodegenerative diseases can utilize insulin and other adjuvant therapeutic agents- this is the main transportation route bypassing the BBB (figure 5),
7. From the CSF pool, the therapeutic agents spread to subarachnoid space around the spinal cord due to CSF circulation and is distributed to the neuropile and neurons of the spinal cord,
8. The therapeutic agents spread to the brain structures and neuropile from the CSF and subarachnoid space, through the Virchow-Robin space deep into cortical centers and spinal cord,
9. From BCSF route of the choroid plexus, the therapeutic agents spread to the ventricle, central canal of the spinal cord and neuropile close to the ependymal lining from the systemic absorption through the respiratory nasal mucosa,
10. Valveless Batson plexus of veins[31] may uptake minuscule therapeutic agents from lamina propria, and transport to the cavernous sinus, other venous sinuses, and to CSF,
11. BV (Batson plexus) and nerve root filaments on the medial walls of the ethmoid air sinus adjoining the olfactory mucosal lamina propria, may transport therapeutic agents,
12. Lymphatics play no role in transport of the therapeutic agents delivered to olfactory mucosa. They only pick up the therapeutic agents or particulate mater leaked through the olfactory nerves at lamina propria from olfactory mucosa (figure 2B, 5).

Olfactory mucosal transport of brain eating amoeba and meningococcus

Evidence of sub perineural epithelial spread of therapeutic agents through the olfactory nerves from olfactory mucosa is further substantiated by the "brain-eating amoeba" which is also transported through the olfactory mucosa, not by the trigeminal ethmoidal nerve. This large10-20 µm in diameter parasitic infections occur when water containing N. fowleri is inhaled through the nose, that comes in contact with the olfactory mucosa.[24] This large celled amoeba, by wiggling its flagella, journeys between receptor and supporting cells spaces (figures 2-6,) and through the spaces created by dying receptors cells reaches CSF and then to brain. It does not pass through the axoplasmic apread or cribriform plate of the ethmoid bone, blood vessels, lymphatics or trigeminal ethmoidal sphenopalatine ganglion nerves. Meningococcus follows the same route, so does also many microorganisms and viruses. Receptor cells axoplams, their axons, olfactory bulb glomeruli do not play any role in this amoeba and meningococcal transport, as opposed to the rabies and other neurotrophic virus spread.

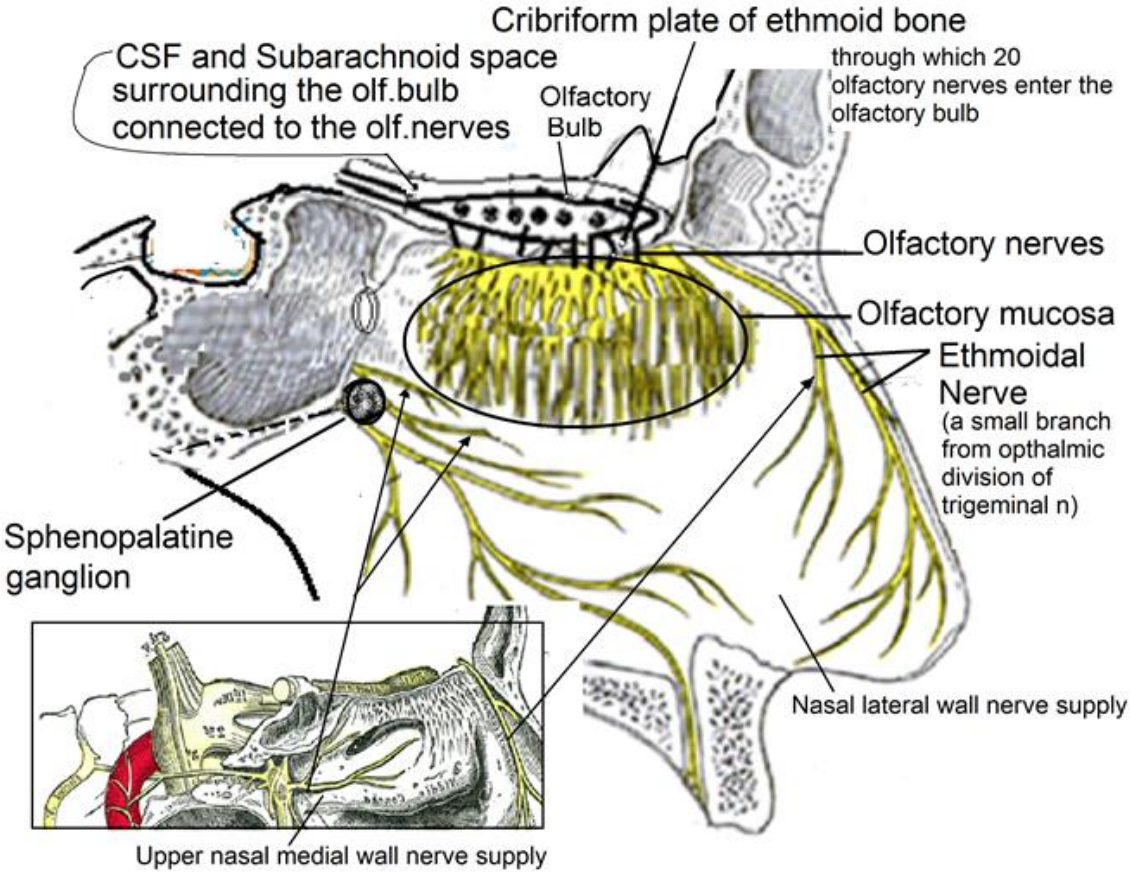

Figure 1 shows the olfactory mucosa and other nerve structures on the walls of the nasal cavity. Note the olfactory mucosa (circled) is the solitary structure that is directly exposed to therapeutic agents, microorganism and amoeba that are conducted rapidly to the CNS by 20 olfactory nerves to the olfactory bulb from where they are transported to the CNS, bypassing the BBB. The (modified from Gray's Anatomy).

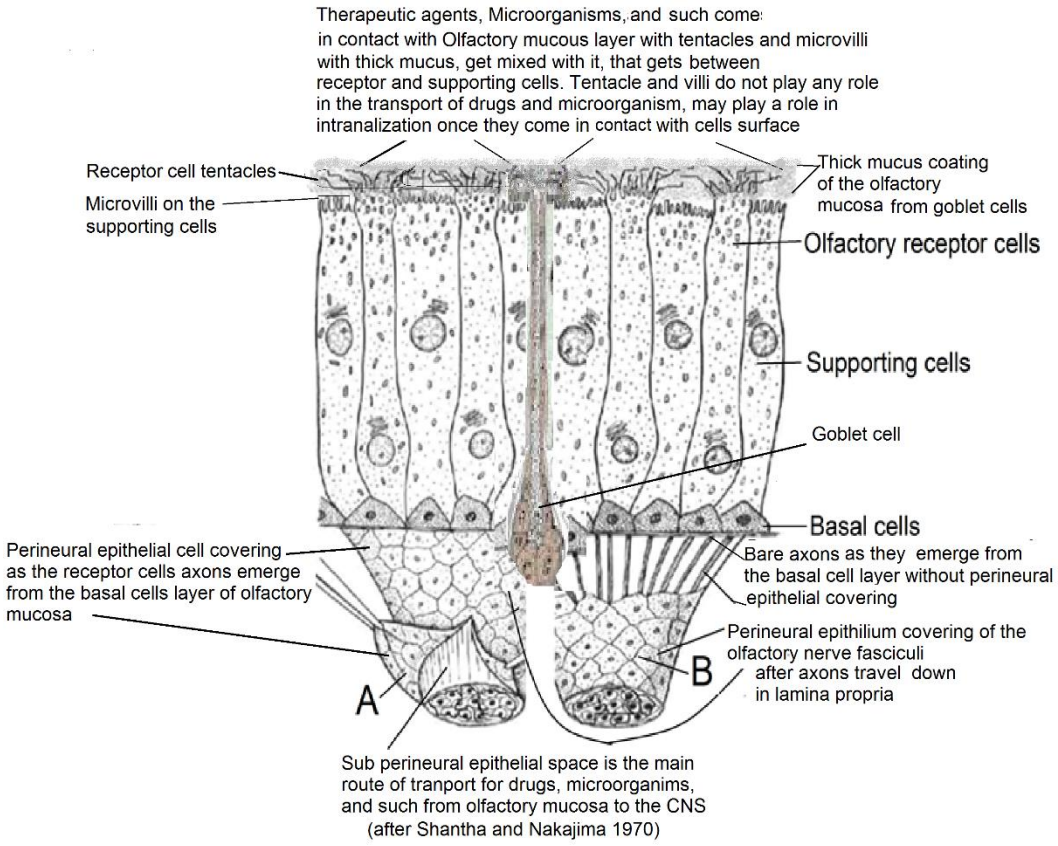

Figure 2 shows the olfactory mucosa and olfactory nerves and their perineural epithelial covering forming the sub perineural epithelial space that transports therapeutic agents and such to the CNS bypassing BBB. Note some of axons bundles are not covered by the perineural epithelial cells as shown in figure B, as they emerge from the basal cells, for a short distance and then get grouped and sheathed by perineural epithelial cells which allows the CSF and therapeutic agents to leak to lamina propria, and picked up by lymphatics (from Shantha and Nakajima 1970).

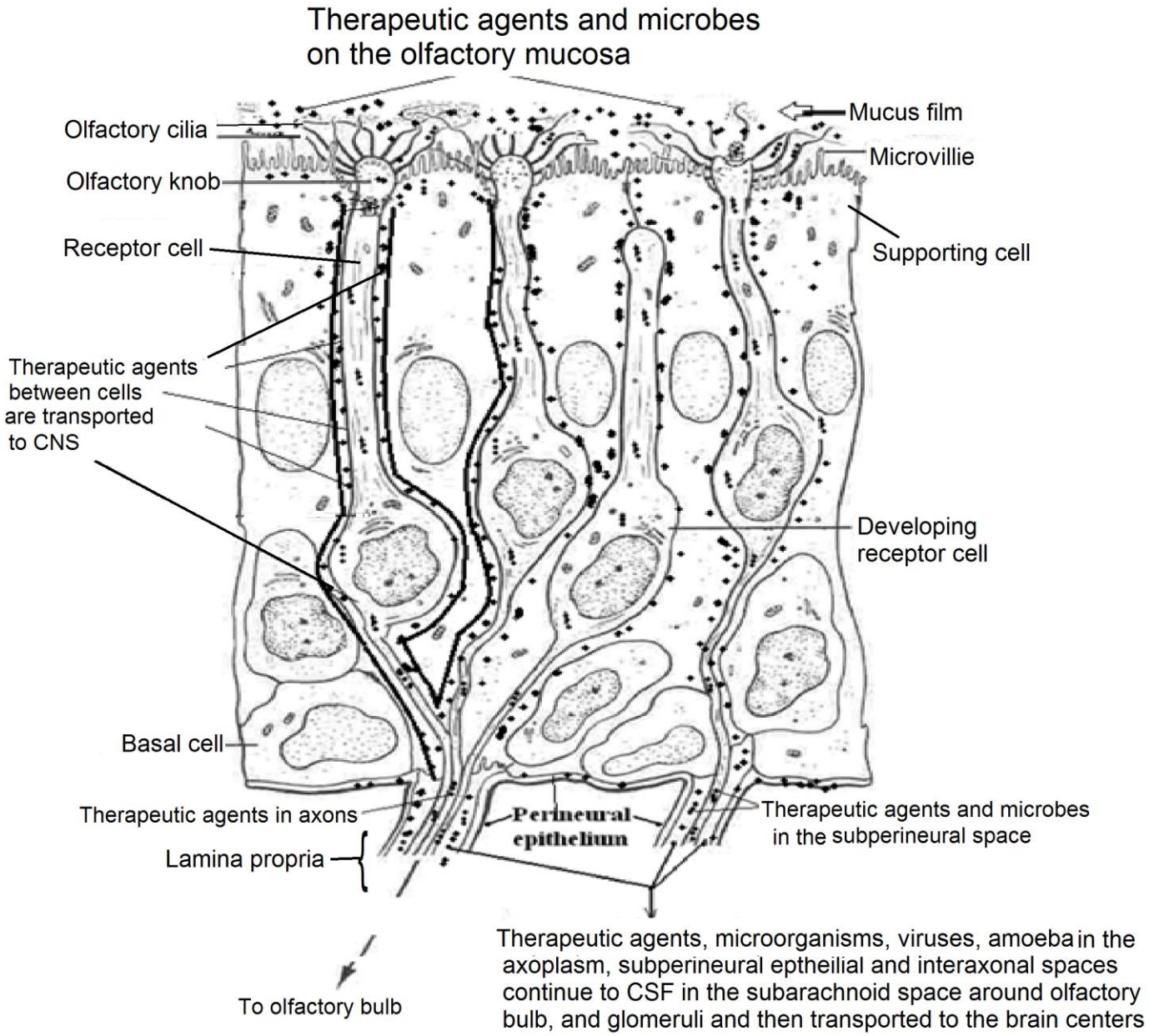

Figure 3 shows the therapeutic agents and microbes deposited on the olfactory mucosa and transported through the axons (intracellular axoplasmic spread) and between receptor cells (para cellular, transcellular, intercellular spread) to the olfactory nerves and under their covering of the 20 olfactory nerves to the olfactory bulb and CSF in the subarachnoid space surrounding the olfactory bulb and to the rest of the brain, bypassing the BBB.

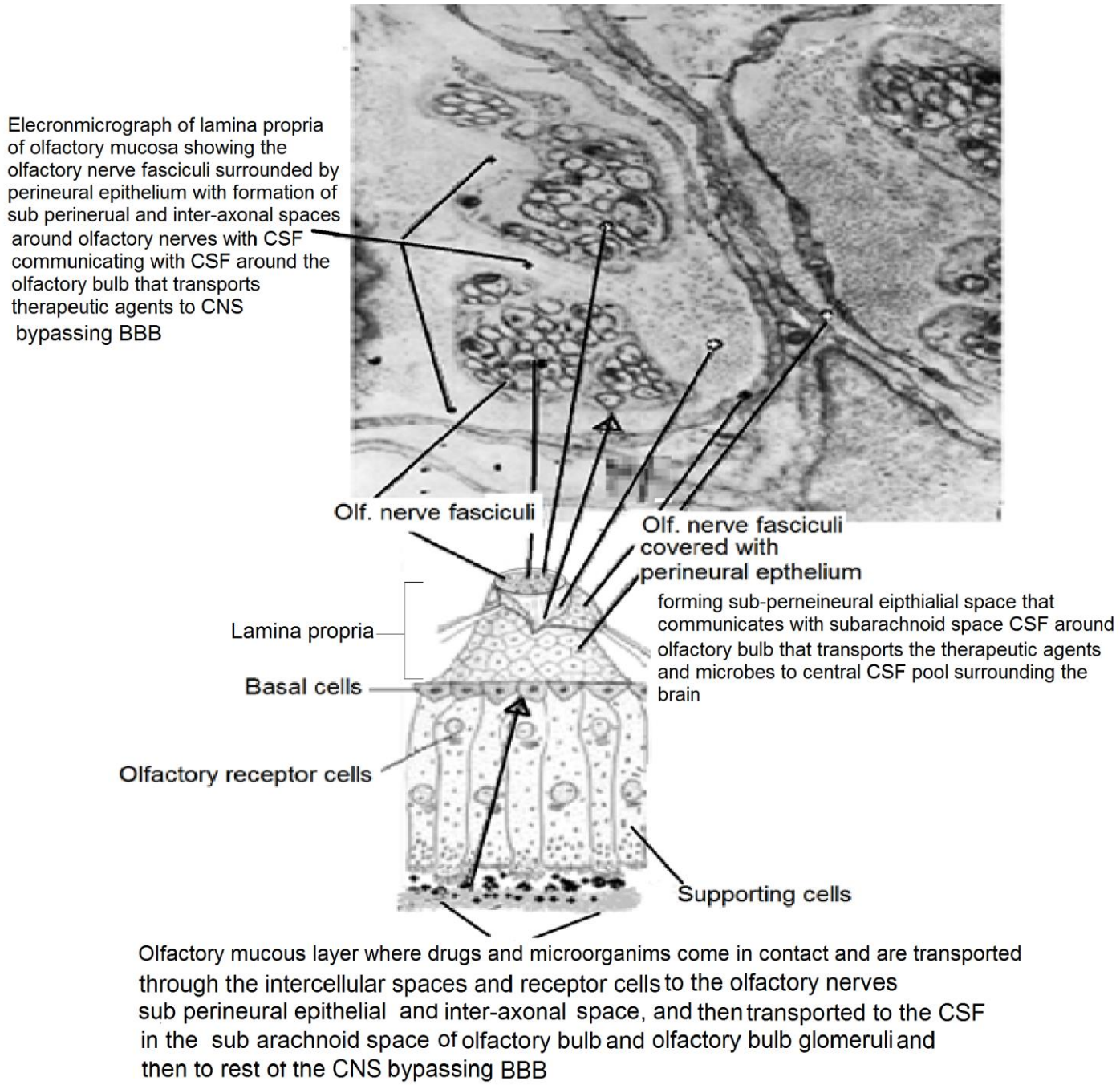

Figure 4 shows the olfactory mucosa with therapeutic agents, olfactory nerves and electron micrograph of olfactory nerves showing the sub perineural epithelial and inter-axonal spaces around the olfactory nerves that transport the therapeutic agents rapidly to CSF around the olfactory bulb sub arachnoid space and to the rest of brain, bypassing BBB as shown in diagram 7.

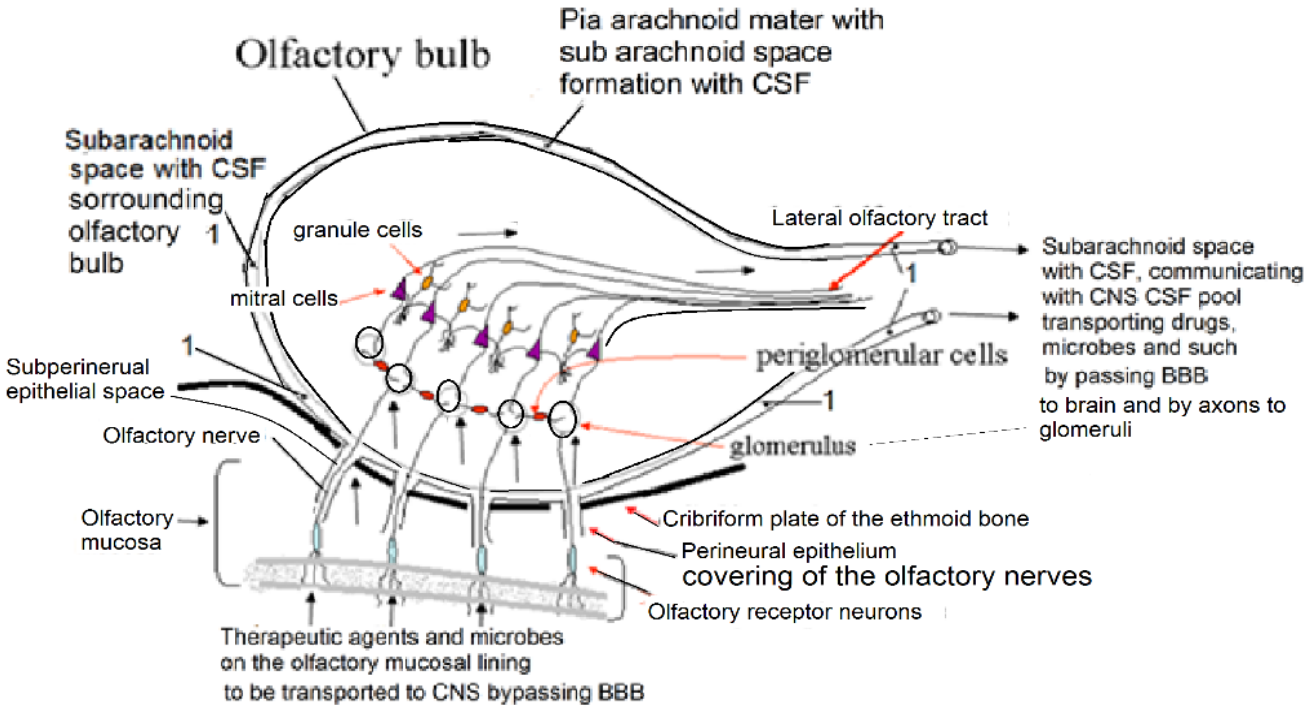

Figure 5 shows the olfactory mucosa, olfactory nerves, olfactory bulb with axonal connection at the glomeruli, and to CSF in sub perineural epithelial space, the sub arachnoid space of the olfactory bulb, olfactory pathway transporting the therapeutic agents, microorganisms and such to the CNS, bypassing the BBB. [13-16]

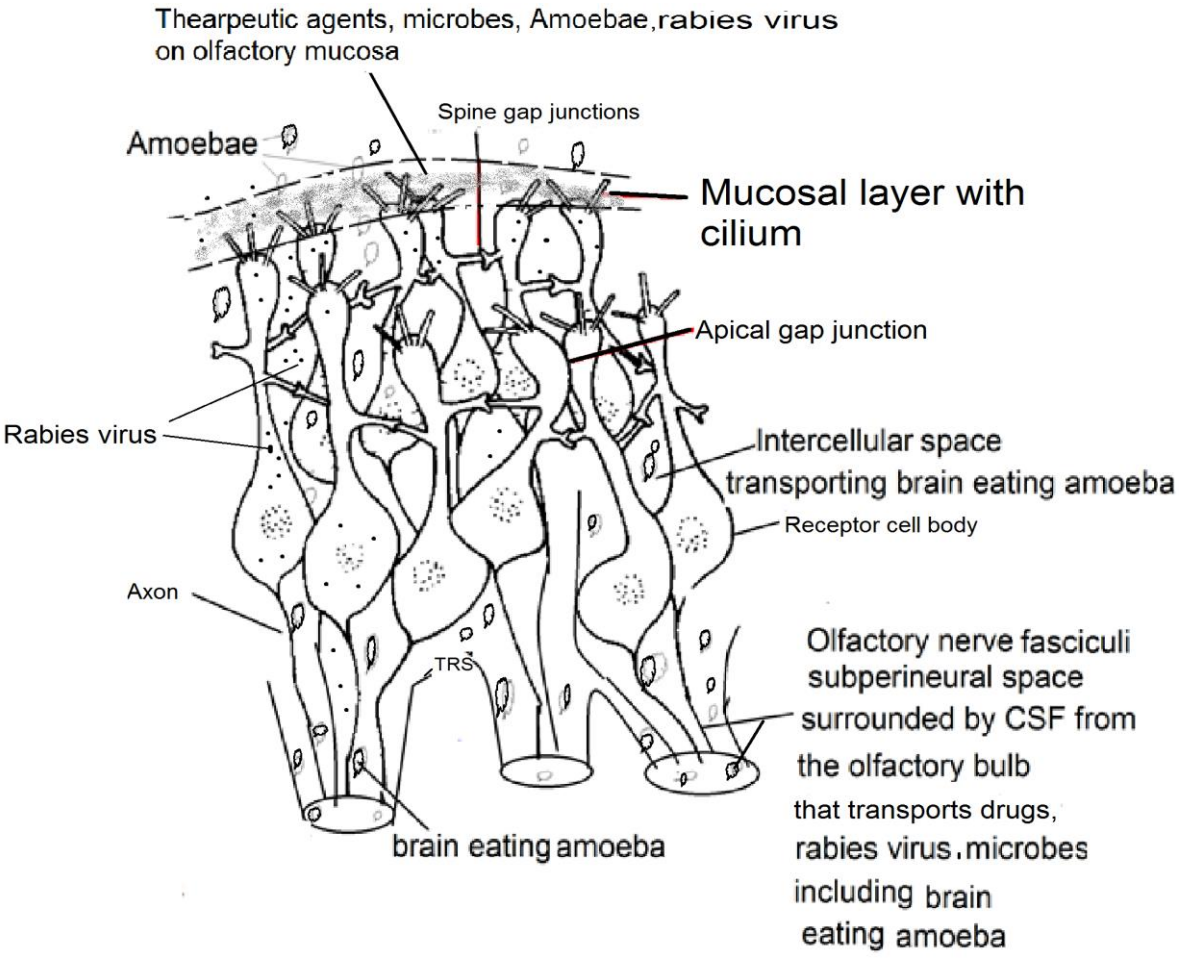

Figure 6 shows the spaces and connections between the olfactory mucosal receptors cells that allow the therapeutic agents, microorganisms, viruses and brain-eating amoeba transported to the CNS through olfactory nerves to olfactory bulb and CSF through these intercellular spaces (diagram modified from Graziadei 1971).

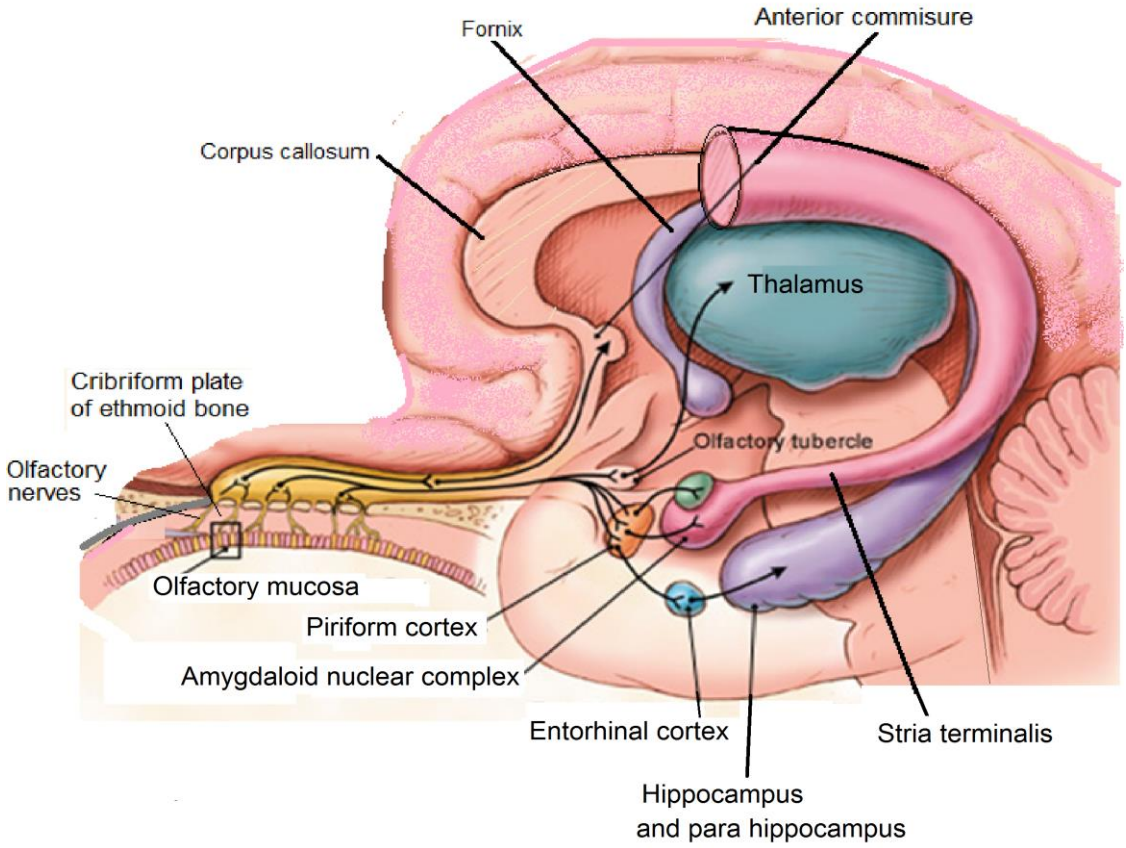

Figure 7 shows the route of transport of therapeutic agents, bacteria, viruses, brain eating amoeba from the olfactory mucosa to various memory centers (labeled) and other areas of the brain, bypassing the BBB through sub perineural epithelial and inter-axonal spaces to CSF content around the olfactory bulb and subarachnoid space as well as through the axoplasmic spread of the olfactory nerves axons to olfactory bulb and connected brain centers.

Trigeminal nerve branch as route for therapeutic agent's transport bypassing BBB from olfactory mucosa

Our studies showed that the only branch that is exposed in the olfactory mucosal region is anterior ethmoidal nerve, a branch of ophthalmic division of trigeminal nerve, and a small fasciculus branch from the sphenopalatine ganglion, not the entire trigeminal complex as publicized. [1-8, 22, 25] These small nerve fasciculi are covered with various connective tissue layers (epi and perineural connective tissue) and multiple layers of perineural epithelial cell with sub perineural epithelial and inter-axonal potential spaces that communicate with subarachnoid space CSF of the CNS and spinal cord (figures 8, 9) that has the potential to transport therapeutic agents to CNS CSF. [13, 15, 16]

The trigeminal branches supplying the nose does not constitute more than 5% of the total trigeminal nerve complex. Our study showed that the subarachnoid space extends on the proximal half of the ganglion. So any pharmacological agent transported through the trigeminal nerve sub perineural epithelial space and inter-axonal spaces ends up mixing with CSF around the ganglion and delivered to pontine CSF cistern. It is not a direct path like the olfactory nerves sub perineural epithelial and olfactory bulb subarachnoid space with flow of CSF, it is a capillary space with slow flow taking many hours and days to reach the central subarachnoid space to exert their therapeutic effect. The therapeutic agents are transported by axoplasmic spread of these nerves and end up spreading to extensive nuclear masses in the brain stem as is the case with rabies. By the time the therapeutic agents reach the nerve fasciculi (figures 8, 9) through diffusion and by Virchow-Robin space [14] of perineural epithelium, it is picked up by the rich blood vessel network of the nasal respiratory mucosa [32] and coverings of the nerve fasciculi before it can be transported in any

appreciable amount bypassing the BBB to reach the CSF around the brain.

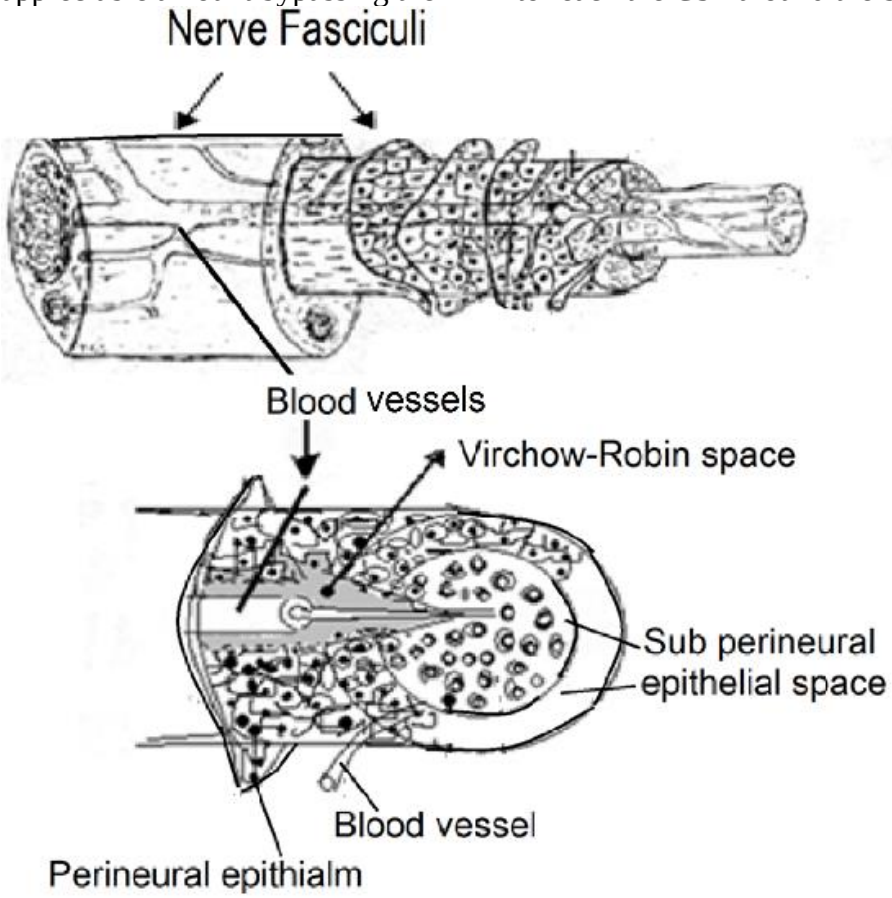

Figure 8 showing the Virchow-Robin space and the covering of the trigeminal nerve fasciculi (from Shantha [13-17]) that transports therapeutic agents to the sub perineural epithelial, and inter-axonal spaces space around the axonal bundles after intranasal administration. Besides the Virchow-Robin space, therapeutic agents also are transported across perineural epithelial and inter-epithelial membrane by diffusion to reach the sub perineural epithelial space of nerve fasciculi.

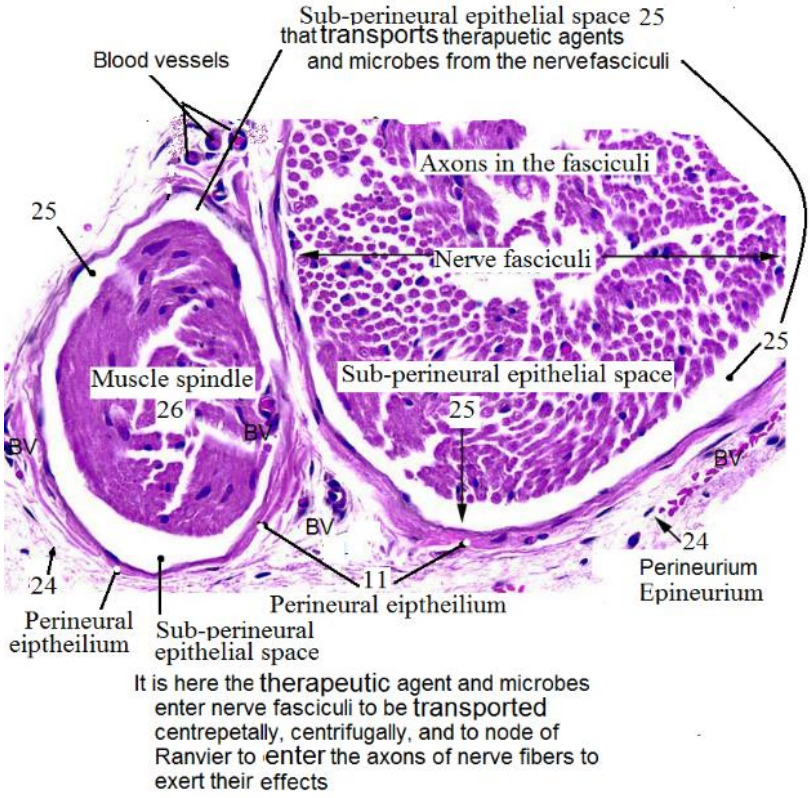

Figure 9 shows the cross section of the trigeminal nerve fasciculi with perineural epithelial covering, [13-16] creating a potential sub perineural epithelial and inter-axonal spaces where the therapeutic agents and rabies virus[26] and such enter to be transported by axons and the CSF to the CNS, bypassing the BBB. Compared to olfactory mucosa, not much therapeutic agents are transported through the trigeminal complex by passing BBB.

Conclusions

We conclude that the 10 square centimeter olfactory mucosa, 20 short olfactory nerves (shortest first cranial nerve) and olfactory bulb play the most important role in transport of therapeutic agents to the CNS, bypassing the BBB not the trigeminal route. We believe that the olfactory mucosa, olfactory epithelium, olfactory nerves, sub perineural epithelial and inter-axonal spaces, olfactory bulb, and the olfactory bulb surrounding CSF, olfactory tract along with suprachiasmatic cistern and inter peduncular cisterns are the essential highway for direct transport of transportable therapeutic agents, microorganism, viruses, and amoeba to the CNS, bypassing the BBB. There is a constant seepage with retrograde and downward flow of CSF from the olfactory bulb to lamina propria, lamina propria lymphatic, BV, and olfactory mucosa itself and vice versa. [1-5, 13, 17, 16, 22, 25] Because some of the merging olfactory nerves axons are not covered by perineural epithelial covering (figure 2B) resulting leakage of therapeutic agents,[16] offending agents from the olfactory mucosa and the central CSF to the lamina propria that are picked up by the lymphatics from the lamina propria draining to the deep cervical lymph nodes. Intranasal olfactory mucosal administration of therapeutic agents for the treatment of neurodegenerative and many CNS diseases overcomes the limitations due to the BBB, and provides an effective method for a selective group of therapeutic agents to be delivered to treat the brain regions that are pathologically affected with Alzheimer's, Parkinson's disease and such.

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