Site of entry of the Rabies virus from the nose and oral cavity; and new methods of treatment of Rabies using olfactory mucosa and by breaking BBB.
To be presented at

RABIES IN ASIA CONFERENCE (RIACON)
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What did the above studies show

- Sciatic Nerve section before rabies virus injection prevents its spread
- Cutting nerve 10 hours after injection does not prevent the spread, before it does prevent the spread
- Crushing the nerve, Wallerian degeneration does not prevent the viral spread indicating intact axons are not needed for viral spread
- Injection of rabies antiserum around and underneath the perineural epithelium did not prevent the spread of the virus.
- Removal of the perineural epithelium slows down the viral spread but does not spread
- "These findings once again point to the Schwann cells, the endoneurium, or associated tissue spaces as the principal progression routes for rabies virus"
- It is interesting to note the complete lack of inflammation in any of the infected sciatic nerves even though viral titres in some nerves was high as we observe in most rabies infected brains.
- Based on our studies over 4 decades, we want to explore how the rabies virus gets into nerve fasciculus, by inhalation, oral routes.
Medial (lateral) walls of the nose with nerves and blood vessels as the route of Rabies virus and bacterial entry into the brain
Medial wall of the nose with olfactory and other nerves as the route of Rabies virus (bacterial) entry into the brain, and SAS (Fig. modified after Grays Anatomy)
Figures I.-4: Olfactory mucosa of Rhesus monkey showing Succinic dehydrogenase, Lactic dehydrogenase, Aldolase, and Glucose-6-phosphate dehydrogenase preparation showing very strong positive activity in the dendritic process of receptor cells Z. Zellforsch. 103. 291—319 (1970). The mucus coat is very lightly positive and Rabies virus CAN sticks to this mucus lining
Olfactory Mucosa and nerve fasciculi of the Rhesus Monkey based on our studies to show how the olfactory axons are surrounded by perineural epithelium as they travel to the olfactory bulb through the cribriform plate of the Ethmoid bone (Shantha & Nakajima Z. Zellforsch. 103. 291—319, 1970)
Olfactory nerves surrounded by Perineural Epithelial cells and route taken by the rabies virus from the olfactory mucosa to enter the olfactory bulb through the axons and sub perineural epithelial space to SAS.
Olfactory nerve fasciculi surrounded by multiple layers of Perineural Epithelial cells which acts as a route taken by the rabies virus from the olfactory mucosa to enter the olfactory bulb and subarachnoid space CSF.
Olfactory nerves surrounded by Perineural Epithelial cells layer which acts as a route taken by the rabies virus from the olfactory mucosa to enter the olfactory bulb.
Olfactory Mucosa with rabies virus or other infecting agents on the surface sticking to the mucus coating and their passage to the brain through axons and subperineural spaces and BV (diagrammatic)
Perineural Epithelial cells covering olfactory nerves continuing with pia-arachnoid mater covering and SAS of the olfactory bulb which can acts as passage for the transfer of rabies virus from the olfactory mucosa.
Complex Glomeruli of the olfactory bulb like a massive distribution stop though which rabies virus reaches the olfactory tract and brain

Glomeruli are made up of
1. Axons from olfactory neurons
2. Mitral cell dendrites
3. Tufted cells
4. Granule cells
5. Periglomerular cells

Centrifugal fibers from
1. Locus Coeruleus
2. Ralphpie nuclei
3. N. Diagonal band of Broca
Routes of Rabies virus spread to central Nervous System From the Olfactory bulb connections and SAS - CSF (modified from Guyton)
Rabies viral Spread from Subarachnoid space after entry into olfactory system (after Shantha)
The vasculature of the hypothalamic median eminence, infundibulum, and the rest of the hypophysis cerebri through which rabies virus can spread if there is viremia (from Gray’s anatomy and Netter Ciba symposium).

Hematogenous spread of rabies virus through Circumventricular organ (Citation: Preuss MR, et al. (2009) PLOS Pathog 5(6): e1000485. doi:10.1371/journal.ppat.1000485)
Circumventricular Organs which can leak rabies virus to the surrounding neuropile and CSF; and their role in spread of Rabies Virus

There are several areas of the brain known as "circumventricular organs". where the BBB is weak which allows substances to cross into the brain somewhat freely. The circumventricular organs include:

- **Pineal gland**: Secretes melatonin and neuroactive peptides. Associated with circadian rhythms.
- **Neurohypophysis (posterior pituitary)**: Releases neurohormones like oxytocin and vasopressin into the blood.
- **Area postrema**: "Vomiting center": when a toxic substance enters the bloodstream it will get to the area postrema and may cause the animal to throw up. In this way, the animal protects itself by eliminating the toxic substance from its stomach before more harm can be done.
- **Subfornical organ**: Important for the regulation of body fluids.
- **Vascular organ of the lamina terminalis**: A chemosensory area that detects peptides and other molecules.
- **Median eminence**: Regulates anterior pituitary through release of neurohormones
- To these I would add **Ependymal lining** of the ventricles and central canal, **choroid plexus, arachnoid villi, pia mater** of the brain and spinal cord and **emerging nerve roots** of the CNS and Spinal cord.
Taste Bud in the Tongue through which rabies virus can enter axons and pass on to brain (after Guyton)
Taste bud with pore, cilia projections, mucus coating at the pore to which rabies virus can get attached. The taste nerve fibers then convey them to brain (Grays Anatomy)
This color-enhanced SE image depicts a taste bud on the tongue. The human tongue has about 10,000 taste buds that are involved with detecting salty, sour, bitter, sweet and savoury taste perceptions. The rabies virus can enter the taste buds through these pores and then passes on to the taste conveying nerve fibers to the brain stem.

- Image: [David Gregory & Debbie Marshall, Wellcome Images](#)
Taste bud, pore, mucus coating, nerve supply and its perineural epithelium coverings based on our studies which can acts as a route taken by rabies virus to enter the CNS. (Shantha and Bourne, _vagus_ Acta Anat 52:95-100)
Electron micrograph of Taste bud base showing the PE cells covering of the nerve fasciculi as they as they emerge from the taste buds.
Central Nervous System connections from the taste buds from which rabies virus can spread to enter brain stem nucleus tractus solitarius through V, VII, IX and X cranial nerves (Diagram From Guyton)
Nerve fasciculi and axonal coverings based our studies spanning 15 years. Perineural epithelium plays a role in spread of rabies virus - it delays virus entry into nerve fasciculi (Baer et al, Shantha and Bourne)
Perineural Epithelium with blood vessel supply to nerve fasciculi which can carry rabies virus to the axons
BV as it enters nerve fasciculi and the Virchow-Robin space around the BV which can also transmit rabies virus to nerve fasciculi interior from outside the nerve fasciculi from the interstitial tissue.
Virchow-Robin Space in Peripheral nervous system which can conduct the rabies viruses to nerve fasciculi interior (Shantha T.R. perivascular Virchow-Robin space in peripheral nerves. V 17, #15, Jan-Feb-Supple, Regional anesthesia, 1992)
EM of Cross section of rat Sciatic nerve and sympathetic chain NF showing various types of NF and node of Ranvier where rabies virus can easily enter the axons (SFNT1968)
A three-dimensional reconstruction of the architecture of intestinal villi and subjacent wall through which rabies virus can enter the Auerbachs and Meissner plexus (Modified From Gray’s anatomy).

There are 130,000 plasma cells /cmm in Lamina Propia of Intestines
Transmission electron micrograph of the columnar epithelium lining the murine small intestine, showing a mucus-secreting goblet cell between two absorptive enterocytes cells which bear microvilli. The cells rest on a delicate basal lamina deep to which is the vascular lamina propria. (Magnification 4,800.) (Prepared and photographed by Mr. Derrick J. Lovell, Department of Anatomy, Guy’s Hospital Medical School, Note the rabies virus can easily enter deeper layers of intestines without coming in contact with brush border of enterocytes.

**Rabies virus can get attached to the surface of the goblet cells and enter the deeper layers of the intestines nerve plexus & BV.**
Rabies virus entry into myenteric nerve plexus in the intestines

7.27A A diagram of an autonomic neuromuscular junction between a group of non-myelinated axons (above) and smooth muscle cells (below). The Schwann cell (blue) is reflected at intervals to expose enlargements of the axons (yellow) which contain synaptic vesicles.

7.27B Electron micrograph of a group of autonomic axons in the myenteric plexus, showing profiles with large dense-cored vesicles typical of the putative purinergic system (see text); smaller, catecholamine-type vesicles are also visible (lower left). Magnification ×40,000.
Diagram showing the entire autonomic nervous system is covered by Perineural epithelial cells under which rabies viruses can easily enter the spinal cord and CNS

(From Shantha and Bournezeitschritc fur Zellforschung 61:742-753 (1641).)
Treatment Outlines:
1. Post Exposure Prophylaxis (PEP);
2. Treatment of full blown Rabies

The following CDC reports available on Internet describes prophylaxis and treatment


Rabies Cases survival and Ketamine treatment: success and failures

- Five cases of rabies have survived so far. They all had Post exposure Prophylaxis (PEP) and intensive care.
- One patient of bat rabies in US survived (Willougby et al 2004). This patient did not receive PEP. She was treated with Ketamine and other antiviral therapies with critical care. There are reports of 3 other cases which survived in a similar treatment (CDC personal communication).
What was the denominator in those who survived from rabies?

Survival of Rabies patients impinges on the early appearance of rabies virus neutralizing antibodies in serum and CSF (Watson et al., 2007 immune responses after rabies infection. Arch. Neurol. 64, 1355—1356).

To survive, rabies virus neutralizing antibodies presence in the CSF is a must.
Where do we go from here?

• I do believe that one day we will have a cure for human rabies. "SEEK YOU SHALL FIND" (MATT 7:7) still holds good in all phases of our research and life; we need to keep seeking answer to this dreaded deadly disease.

• I do believe that there will be development of effective (with least untoward reaction), one or two shot prophylactic vaccine to be used in PEP.

• I want to introduce today the method of treatment which I do believe can be effective and save some if not all rabies patients.
The principle methods I propose to treat Full Blown Rabies Cases are (patented)

1. Intranasal administration of antirabies therapies including Monoclonal antirabies and anti TNF MAB (Etanarcept) to reduce the brain inflammation using insulin as enhancer of uptake from olfactory mucosa and carry them to the brain.

2. Antirabies therapy through subarachnoid space with insulin

3. Intravenous or intra-arterial antirabies therapy after BREAKING THE BLOOD BRAIN BARRIER to get it into the substance of the brain with insulin pretreatment to enhance the NEUROPILE uptake.

4. Intraventricular delivery of antirabies therapies with insulin.

5. Enhance the immune system by orally administering immune stimulating component of the rabies virus and / or other such immune enhancer through NG tube or Gastro-jeunostomy tube (or IM with insulin) to stimulate the plasma cells(180,000 /cubic mm) in the lamina propria of the intestines, to produce neutralizing rabies antibodies.

6. Lower the metabolism of the brain by lowering the blood sugar level, what I call INTERMEIDATE HYPOGLYCEMIA every two to 6 hours once to enhance the uptake of neutralizing antirabies antibodies and therapeutic agents.

7. Supportive critical care therapy with Critical care monitoring.
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HUMAN ANTIBODIES AGAINST RABIES AND USES THEREOF

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ATTENUATED RABIES VIRUS WITH NUCLEOPROTEIN MUTATION AT THE PHOSPHORYLATION SITE FOR VACCINATION AGAINST RABIES AND GENE THERAPY IN THE CNS

Inventor: Zhen Fang Fu, Athens, GA (US)

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ABSTRACT
A mutant virus is provided which contains a mutation at a

United States Patent
Hooper et al.
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RABIES VIRUS-SPECIFIC NEUTRALIZING HUMAN MONOCLONAL ANTIBODIES AND NUCLEIC ACIDS AND RELATED METHODS

Inventors: Douglas C. Hooper, Medford, NJ (US); Bernhard Dietzschold, Newtown Square, PA (US)


TREATMENT OF RABIES CASES

THERAPEUTIC PLAN- PART ONE

- Intranasal delivery of antirabies antibodies (human MAB), Nerve growth factors, erythropoietin, insulin, Insulin growth factor-I, and other antiviral therapies using insulin as uptake, therapeutic activity enhancer and helper in dissipations therapeutic agents to the CNS to attack the rabies virus

- This is a method of going "through" and / or "behind" the BBB.
Advantages

- Painless
- Ease of use
- No shot needed
- Avoids first pass metabolism – improving bioavailability over oral and rectal doses
- Nose-brain pathway allows direct delivery to the cerebral spinal fluid
- Compliance not an issue - easy and fast to deliver to any patient

Disadvantages

- Limited medications that can be delivered in this fashion
- Many medications are not adequately concentrated to achieve ideal dosing volumes
- Mucosal health impacts absorption

How administered

Ideally, drug doses will be divided in half, and each nostril received half the dose, which doubles the absorptive surface area.
Intranasal olfactory mucosal delivery of Antirabies therapies

1. Rapid appearance of 1 ‘25-labeled nerve growth factor in the olfactory bulbs, cerebrum, and brain stem is more consistent with entry of label through intercellular clefts in the olfactory epithelium and extracellular transport along the olfactory neural pathway to reach the cerebrospinal fluid and brain than with uptake by olfactory neurons and subsequent intracellular axonal transport. (Drug Delivery, Volume 4, Issue April 1997, pages 87 – 92)

2. Olfactory and trigeminal-associated extracellular pathways to rapidly elicit biological effects at multiple sites within the brain and spinal cord (Neuroscience 2004, 127:481-496)).

3. Kinetic studies showed that intranasally administered plasmid DNA reached the brain with a 2,595-fold higher efficiency than intravenously administered plasmid DNA (J Mol Med. 2007 Jan;85(1):75-83. Epub 2006)
Intranasal – olfactory delivery catheter balloon device (Shantha-patent pending)
1. Going through the BBB by Breaking the Blood Brain Barrier and deliver therapeutic agents against rabies virus. (By use of endogenous transport systems, and carrier-mediated transporters such as glucose and amino acid carriers; receptor-mediated transcytosis for insulin or transferrin; and blocking of active efflux transporters such as p-glycoprotein. USE OF INSULIN AND INDUCTION OF INTERMEDIATE HYPOGLYCEMIA DESCRIBED IN OUR PATENTED METHOD INCORPORATES MANY OF THESE PRINCIPLES).

2. After breaking the BBB, the antirabies viral therapy can be administered IV and / or Intra arterial method by directly infusing internal carotid arteries on both sides to deliver into the brain.

3. By linking transferrin with rod-shaped semiconductor nanocrystals (quantum rods) and Nanoparticles-based platform which 'tricks' the BBB into allowing the entry of the nanoparticle into the brain, using an approach that draws parallel to the 'TROJAN HORSE' concept. They are not yet available.
The BBB can be broken down by:

- **Hyperosmolitity**: a high concentration of a substance in the blood can open the BBB.
- **Vasoactive substances** such as Brandykinins opens BBB.
- **Insulin** enhances the transfer of therapeutic agents from the BBB, and membranes covering the nervous system to neuropile.
- **Hypertension**: high blood pressure opens the BBB.
- **Developmental**: the BBB is not fully formed at birth. **Microwaves**: exposure can open the BBB.
- **Radiation**: exposure can open the BBB.
- **Infectious agents**: exposure can open the BBB.
- **Trauma, Ischemia, Inflammation, Pressure**: can open the BBB.
- **HIFU**: High intensity focused ultrasound opens BBB.
400 mile long Capillary system with astrocytes feet surrounding their wall contributing to BBB which prevent effective anti rabies viral therapeutic agents reaching neuropile.
in the early 1980s, Edward A. Neuwelt, M.D., an OHSU neurosurgeon, pioneered a unique method of outwitting the brain's protective blood-brain barrier. By temporarily opening this barrier, chemotherapy and **Viral - tumor specific antibodies** can pass into the brain and reach the tumor. Dr. Neuwelt continues to devote his efforts to research.

Diagram of blood-brain barrier at cerebral capillary (from Rapoport, 1976)
TREATMENT OF RABIES CASES

THERAPEUTIC PLAN- PART THREE

• Subarachnoid space (SAS) and CSF delivery of antirabies therapeutic agents including but not limited to human antirabies MAB though continuous SAS catheter or injection into Cisterna Magna or close to the SAS at Cervical spine levels using **insulin** as uptake and therapeutic agent activity enhancer and dissipater of the therapeutic agents.
Subarachnoid – CSF delivery of antirabies therapeutic agents including antirabies MAB and ketamine with insulin (continuous or intermittent delivery)
Virchow- Robin space entry of antirabies therapeutic agents with use of Insulin deposited in the SAS-CSF (such a space is also found in peripheral nerve fasciculi 
Shantha 1992 ASRA)

Therapeutic agents can permeate 6mm into brain substance With Insulin, it can still permeate deeper
Subarachnoid and CSF delivery of immune substance and antiviral agents

- Therapeutic agents have hard time entering the neuropile from SAS because the astroglial cells feet attach to the pia mater and which acts as barrier: PIAL BRAIN BARRIER.
- From CSF and Pia, the drugs can penetrate up to 6mm surface of the brain under normal circumstances.
- Some of the therapeutic agents can enter through Virchow-Robin space, but it is minimal and will not be able attack the virus in deeper depth of the brain.
- Our method of use of insulin with therapeutic agents will allow penetration into deeper depths of the brain to eradicated the offending agent at least from the surface of the brain, spinal cord and proximal part of emerging cranial and spinal nerves.
TREATMENT OF RABIES CASES

THERAPEUTIC PLAN – PART FOUR

• Intraventricular injection of therapeutic agents against rabies virus using insulin to enhance their uptake, activity and facilitate distribution through the Ependymal lining using Ommaya or other delivery system.

• This is a method of going "through" and/or "behind" the BBB.

• ***Strategies for drug delivery by intracerebral implantation and convection-enhanced distribution be avoided.
Antirabies therapeutic agents (MAB and other therapeutic agents) administered with Insulin to the ventricles of the brain though a Ommaya reservoir and infusion port using Huber needle

Ommaya reservoir is an intraventricular catheter system used for the delivery of drugs (e.g. chemotherapy, antiviral and antibacterial therapy) into the cerebrospinal fluid. It was originally described in 1963 by Ayub K. Ommaya, a neurosurgeon.
Therapeutic agent uptake of Antirabies virus therapies: from Ependyma to Pia mater after Intraventricular delivery with insulin. Neuralgia are shown in green (from Gray’s anatomy)
• Enhance the immune system by orally administering immune stimulating component of the rabies virus and / or other such immune enhancer through NG tube or Naso- Gstro-jejunostomy tube to stimulate the plasma cells (180,000 cells /cubic mm) in the lamina propria of the intestines, to produce neutralizing rabies antibodies.
• Can inject the antibody stimulating rabies virus component IM, IV, or SQ using insulin to enhance its uptake, distribution resulting in stimulation of viral antibodies by the immune system.
• Start with low doses and increase the dose every day.
TREATMENT OF RABIES CASES

THERAPEUTIC PLAN – PART SIX

• Lower the metabolism of the brain by lowering the blood sugar level, what I call INTERMEIDATE HYPOGLYCEMIA (40-60 mg%) every 6 to 12 hours once to enhance the uptake of neutralizing antirabies antibodies and other therapeutic agents into neuropile and to enhance the uptake, enhance the activity and dissipation of therapeutic agents.
TREATMENT OF RABIES CASES

THERAPEUTIC PLAN – PART SEVEN

• Critical care monitoring as needed and as available – see the publication on the internet about Milwaukee protocol and follow the guide lines they describe.

• Monitor fluid intake and output. Avoid over hydration and maintain the BP enough to perfuse the brain. Use all supportive therapies to maintain heart, lung, and kidney functions.

• Maintain Electrolyte balance

• Patient on respirator should receive enough to maintain 100% oxygen saturation to avoid lung damage.

• Sedation with ketamine and midazolam

• Keep paralyzed if the patient is fighting and having muscle spasms by using non depolarizing muscle relaxants.
Antirabies Viral therapy- Part I

1. ***No medications are given intrathecally (MP)- I am opposed to it. Give small antirabies human monoclonal antibodies (MAB) with other antiviral agents including Ketamine with 3-5 units of insulin pretreatment.

2. ***Avoid rabies immunization of the patient after onset of clinical symptoms (MP)- I am for giving small doses rabies antigen; increasing the dose every day through the Naso- Gastro-Jejunostomy tubes. Intramuscular antirabies immunization can be administered mixed with 1-2 units of insulin for rapid absorption and dissipation to immune system stimulation.

3. Avoid administration of rabies-specific antiserum (MP)- I am for giving intranasal, SAS, and Intracisternal delivery of MAB and other anti rabies viral agents with insulin.
1. **Avoid administration of IFNa** - I am for administering MAB against TNF and cytokines to lower the brain inflammation such as Etanarcept with insulin.

2. **Ribavirin is no longer recommended** (MP) – I will administer small doses intranasal and other routes because it is a prodrug, which when metabolized resembles purine RNA nucleotides. In this form it interferes with RNA metabolism required for viral replication. Why not add qhinine sulfate, it is antiviral?

3. **Amantadine** is administered orally or intranasal & other routes here in described. **It does Passes through BBB and has both anti Parkinson's and antiviral effect. It may block reproduction of the VIRUS and decrease the ability of the virus to get into the cells. Believed to release brain dopamines from nerve endings in Parkinson's.**
Antirabies Viral therapy- Part III

- **Ketamine** is much debated and it is opposed by some rabies researchers due to failure of save rabies patients.
- I would use it intranasal (IN), SAS, Intracisternal, IV or intra arterial routes. Studies show that it acts at the level of rabies virus genome transcription and prevents it multiplication. It is also non a competitive blocker of the NMDA receptors on the nerve cell. It sedates at the same time blocks the virus multiplication. I have used this drug on patients since 1968. It is one of the safest drug. I will use it on rabies patients though the latest studies are disappointing.

1. **IN- Erythropoietin:** rhEPO i.n. reduced infarct volume, brain swelling and cell damage in the ischemic hemispheres, and improved behavioral dysfunction 24 h after cerebral ischemia (Neurosci Lett. 2005 Oct 14;387(1):5-10)
3. **Any latest antirabies antiviral therapy** with insulin uptake, activity and dissipation enhancer.
4. Magnesium can be used to relax synaptic discharge.
5. Provide antipyretic therapy if the body temperature raises.
Antirabies Viral therapy- Part IV
Use of human antirabies MAB

1. Use recombinant fully human anti-rabies monoclonal antibody that specifically binds a broad variety of rabies virus isolates and inhibits the ability of the virus to infect cells and cause the disease or prevent their further spread and pathology by neutralizing them.

2. Advantages:
   - They are likely to be less adverse immunogenic in humans than non-human antibodies.
   - Rabies virus inactivation can occur as soon as the antibody reaches sites of infection.

3. Combining MAB with insulin as enhancer of uptake and therapeutic action, the rabies virus can be effectively neutralized providing a cure for this incurable disease and act as effective PEP therapy.

4. Combining other therapeutic antiviral agents can augment the effectiveness of the antirabies therapy.

5. IMPORTANT: These MAB and rabies antigens should be used with insulin (patented method) as part of pre and post exposure prophylaxis.

6. Use Human monoclonal antirabies antibodies (MAB) intranasal, IV, SAS, Intracisternal methods with insulin as uptake, activity and distribution enhancer.