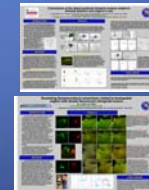


The Neuroanatomy of Hemispatial Neglect: New Methodologies and Implications

Thesis Defense
William L. Conte

Accomplishments

- 4 article submissions
 - 2 in Brain Research (first-author and second-author)
 - Journal of Neuroscience Methods (first-author)
 - Journal of Visualized Research (first-author)
 - Featured on the cover of Brain Research
- 3 posters
 - Two first-authored
 - Two presented at SFN
- 4 research grants
 - 2007:
 - Vetmed University Scholars Program (USP)
 - HHMI Science for Life
 - 2008:
 - Fine Arts USP
 - Vetmed USP (declined stipend)
- Also a member of 2 other research groups
 - Dept. of Neurosurgery: epilepsy and stem cell research
 - Dept. of Musicology: neuromusicology



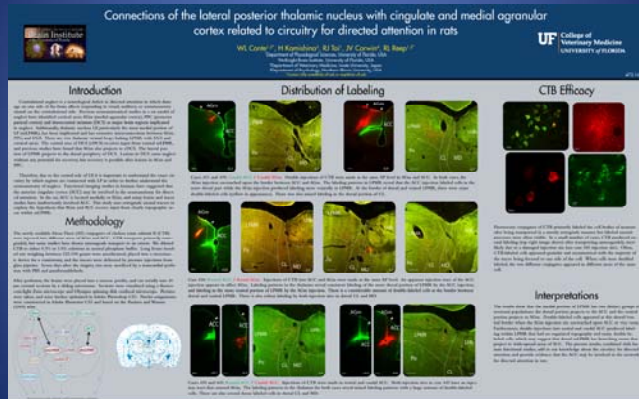
The Future

- Medical School
- MD-PhD Program
- Where? I don't really know yet.
 - Recently accepted to Loyola University Stritch School of Medicine



Background

SFN 2008 Poster

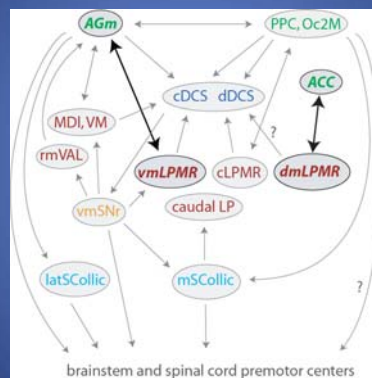


Neglect

- Dysfunction of directed attention
- ~60% of stroke patients exhibit neglect
- Strong neuroanatomical component



Neuroanatomy of networks

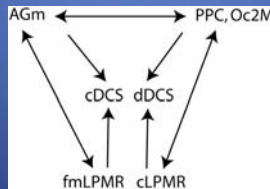


Lesion Models

- Apomorphine injections produce temporary recovery
 - Recovery
 - cortical areas (AGm, PPC)
 - LP
 - No recovery
 - DCS
- DCS thought to be the region that mediates spontaneous recovery

LP

- Plays a central role in the circuitry
- Has connections to both the cortex and the striatum
 - fmLPMR connects with AGm and cDCS
 - AGm also connects with cDCS
 - AGm and LPMR are thought to synapse on the same group of neurons
- Similar findings for PPC, central LPMR, and dDCS



My Research

My Research Focus

- Methodologies
- Thalamus and cortex connections and topography

Tracer methodology study

Submitted to *The Journal of Neuroscience Methods*

DA

- Dextran Amines
 - The most popular neuronal tracer
 - Commonly used in 3k and 10k MW
 - 10k is mostly anterograde
 - 3k is mostly retrograde, but there is often unintentional anterograde labeling
- Buffer type, pH, survival time, and MW affect mechanism of transport

Problem

- Initial motivation for the project: green tracer conjugate was not showing up
- Red and Green conjugates were also unintentionally transporting anterogradely

Results

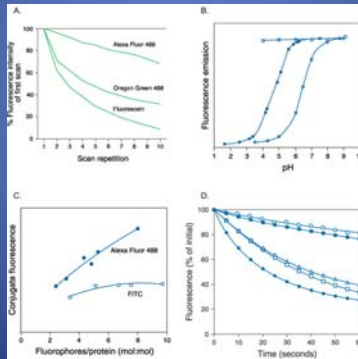
- Failed
 - Never solved the problem of the missing green
 - Theory: fluorescein is not a good dye
 - Another thought: fluorescein does not fluoresce very brightly in acidic conditions
 - No correlation with tracer conditions and mechanism of transport
- Solution:
 - Try a new tracer

CTB

- Cholera Toxin subunit B
 - Viral tracer
 - Mostly retrograde (but sometimes anterograde)
 - Good results using HRP conjugates
- In 2007, Invitrogen attached the Alexa Fluor line of probes to CTB
 - We were the first ones (I think) to use fluorescent CTB in this circuit
 - Challenge: modify HRP protocol for fluorescent tracing

Alexa Fluor

- Very bright
- Very photostable
- Stable at all pH
- 3k DA is not available with AF conjugates (but some 10k are)



CTB Results

- It is a very good tracer
 - More viscous than DA
 - Small injection sites
 - More sensitive than DA
 - Smaller injection sites had more labeling
 - Labeling was brighter
 - Highly photostable
 - Highly specific for retrograde transport
 - Although damaged injection sites produced anterograde labeling

Implications

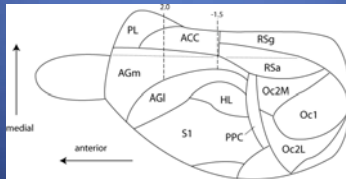
- Cingulate Cortex Study
 - Small injections allowed us more maneuverability
 - Confined injections allowed us to have two injection sites less than 0.5 mm away from each other

Cingulate Cortex

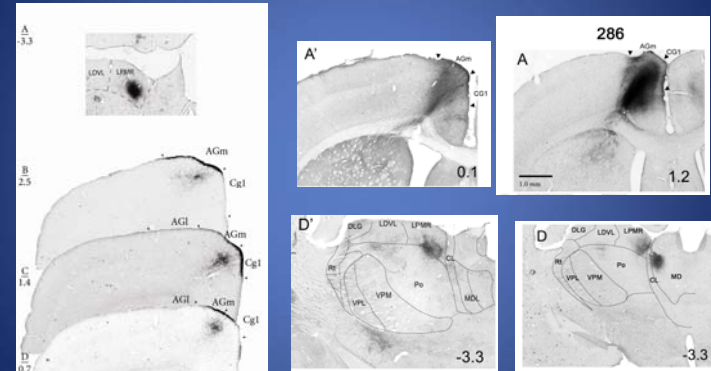
Published in *Brain Research*

Anterior cingulate cortex (ACC)

- Also known as CG1 in the rat
- Thought to be responsible for attentional processing in humans (amongst other things)
- Cingulate lesions in humans produce neglect
- Located medially to AGm
 - Most lesion studies in animals have inadvertently involved ACC
 - Many tracer studies in AGm have also inadvertently involved ACC

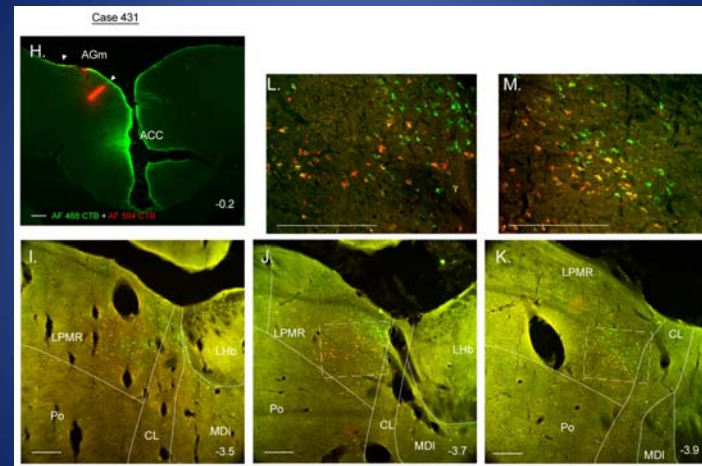
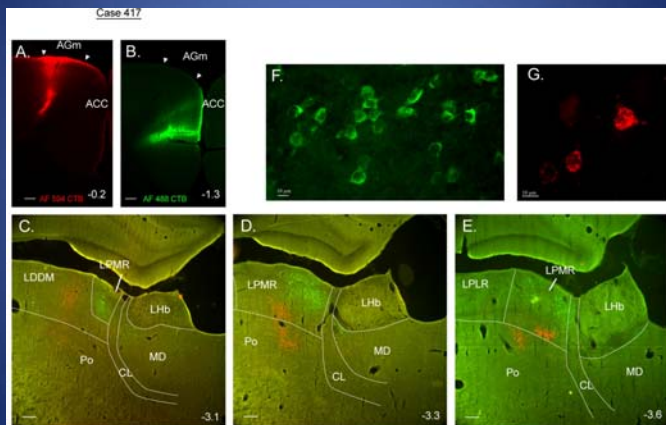


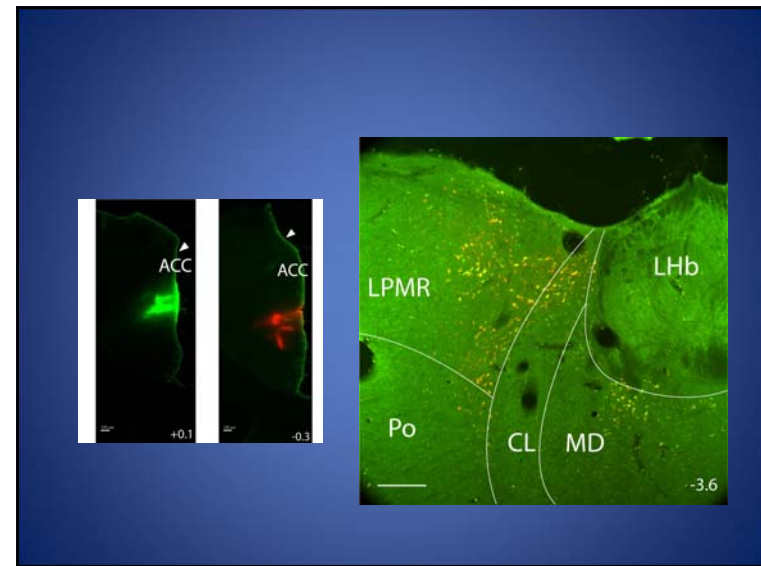
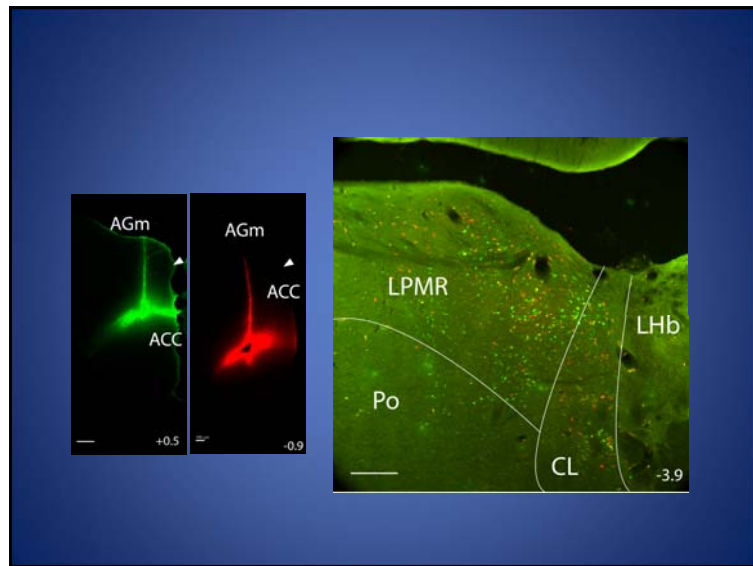
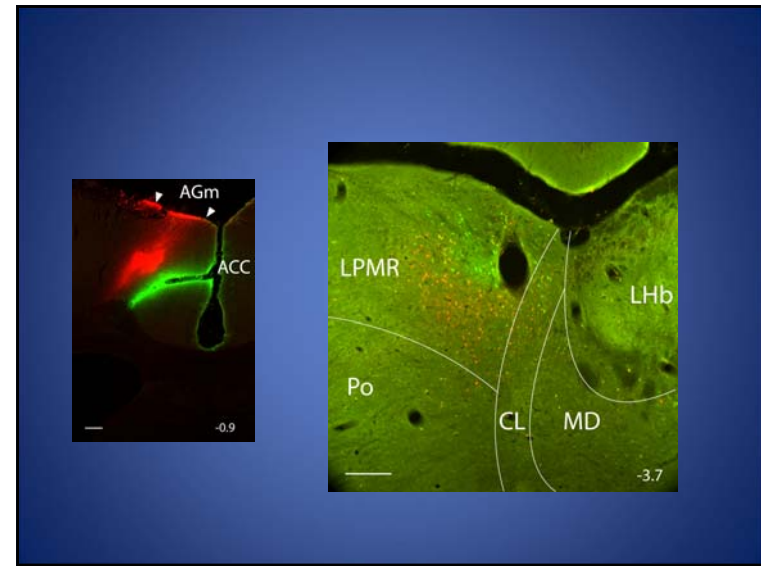
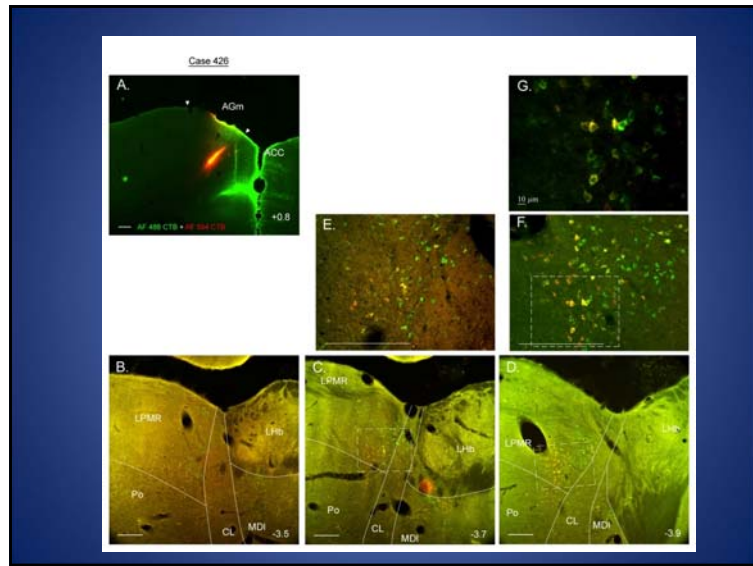
10k DA dorsal/ventral tendencies



Kamishina, Conte, et al., 2009

CTB results





Conclusions

- Dorsal LPMR → ACC
- Ventral LPMR → AGm
- The boundary is not clear

Future directions

- Dorsal vs. ventral LPMR projections to DCS
 - Need double tracer injections in LPMR
- Reciprocal connections between dorsal LPMR and ACC?
- Connections between ACC and DCS?
- Lesion studies