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NYIT College of Osteopathic Medicine

Examining ICC Networks to Study the Effect of Calcification Induced Heart Failure on GI Dysmotility

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New York Institute of Technology College of Osteopathic Medicine, Old Westbury, NY

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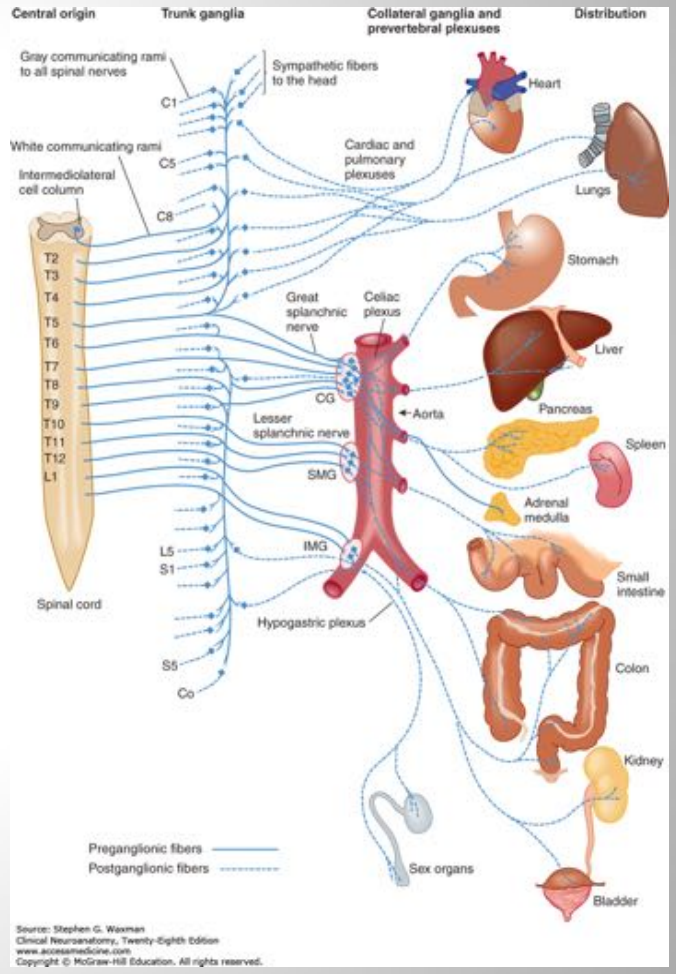
Introduction | Methods | Results | Discussion

- **Over 23 million people in the world are afflicted with heart failure, many of whom suffer from GI symptoms, the pathology of which has not been adequately studied.**
- **In our previous research using a mouse model of increased vascular calcification, we uncovered a link between chronic HF and reduced GI motility**
- **GI motility is controlled by interstitial cells of Cajal (ICC), the electrical "pacemaker" cells of the GI system**
- **We established that mice affected by vascular calcification and HF have reduced slow wave amplitudes with preserved frequency**

- ***We wish to examine whether vascular calcification-induced HF is associated with loss of ICC or the connectivity of ICC networks, to determine whether this plays a role in GI dysmotility of these mice***



This image illustrates that the body works as a unit. Specifically, we should note how the sympathetic chain via branching neurons innervate the viscera, including the heart and GI organs. This connection may be explored when examining heart failure and GI motility.

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Dissection of WT and eTNAP: SI, Cecum and Colon

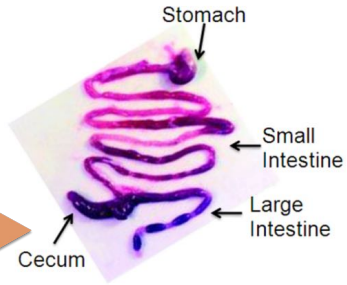
18-23 weeks old

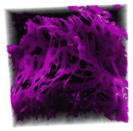
Wild Type

eTNAP
(endothelial Tissue Nonspecific Alkaline Phosphatase)

Echocardiography to confirm Heart Failure

3D schematic of GI Tract:



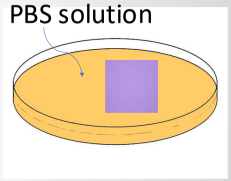


Click here for larger video! 3D reconstruction of ICC Networks:

Chen, Y., Shamu, T., Chen, H., Besmer, P., Sawyers, C. L., Chi, P. Visualization of the Interstitial Cells of Cajal (ICC) Network in Mice. *J. Vis. Exp.* (53), e2802, doi:10.3791/2802 (2011).

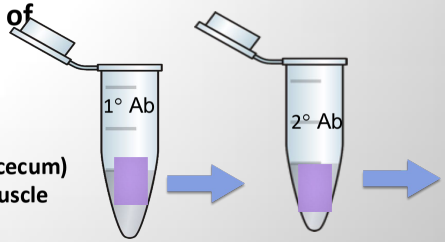
Whole Mount Sample Preparation

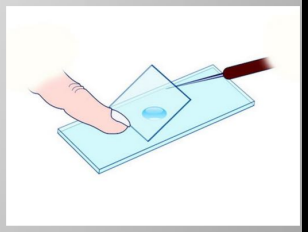
5x5 mm sections of SI, Cecum and Colon were flattened and mucosal layer was scraped off, leaving muscularis layer intact.



c-Kit Immunofluorescent Staining

Samples were stained with rat anti-cKit (primary antibody) and Alexa Fluor 594 anti-rat (secondary antibody). These sections of tunica muscularis of the 2 regions were mounted serosal side up.





Visualization of ICC Networks

Via Olympus BX53 Upright Microscope: Images were viewed under 20x objective and taken with standardized exposure time, and the depth of focus corresponding to ICC-MY in the SI/cecum and ICC- IM in the colon

Regions:
ICC–Myenteric Plexus (MY): responsible for slow wave activity (in SI & cecum)
ICC–Intramuscular (IM): responsible for neuromodulation of smooth muscle cells in GI tract & some slow wave activity in colon

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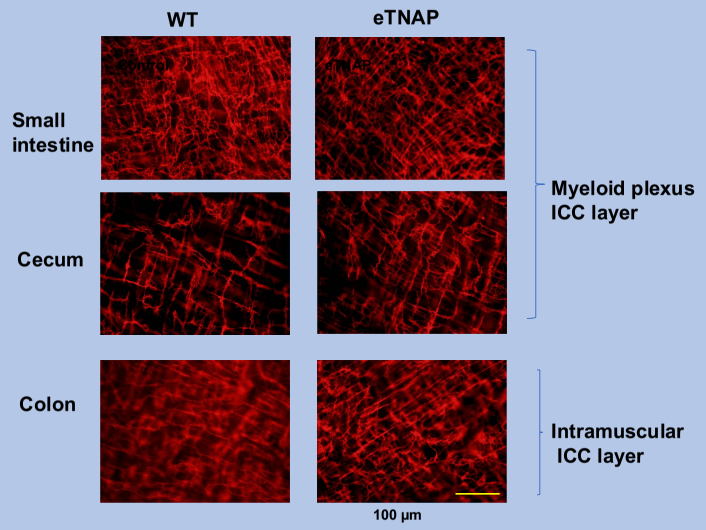
Echocardiography Findings (Mean ± SD)

	Wild Type (WT)	eTNAP	TTEST p value
N	10	9	
BW, g	26.3 ± 0.8	21.6 ± 1.3	< 0.001
HR, bpm	449 ± 51	423 ± 44	0.283
EF, %	57.3 ± 15.9	29.6 ± 18.4	0.004
FS, %	26.6 ± 9.9	12.3 ± 8.9	0.006
LVID/BW, mm/g	0.16 ± 0.01	0.26 ± 0.05	< 0.001
LVPW/BW	0.030 ± 0.005	0.030 ± 0.009	0.986
LVmass/BW	3.6 ± 0.9	5.3 ± 1.7	0.021
CO, ml/min	18.6 ± 6.9	18.7 ± 4.7	0.981

BW, body weight; HR, heart rate; EF, ejection fraction; FS, factional shortening; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall thickness; LVmass, left ventricular mass; CO, cardiac output

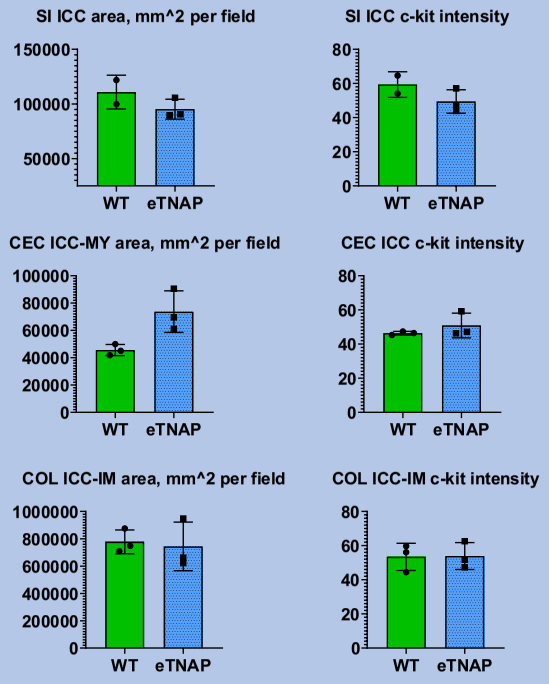
When compared to WT mice, eTNAP mice had reduced body weight ($p < 0.001$), reduced left ventricular (LV) ejection fraction ($p < 0.01$) and increased LV mass index ($p < 0.05$).

Immunohistochemistry



Upon comparing the immunofluorescent images of c-kit antibody stained ICC networks between WT and eTNAP mice, we did not see any prominent changes in the ICC networks in the SI or the colon.

Quantitative Assessment of ICC Networks



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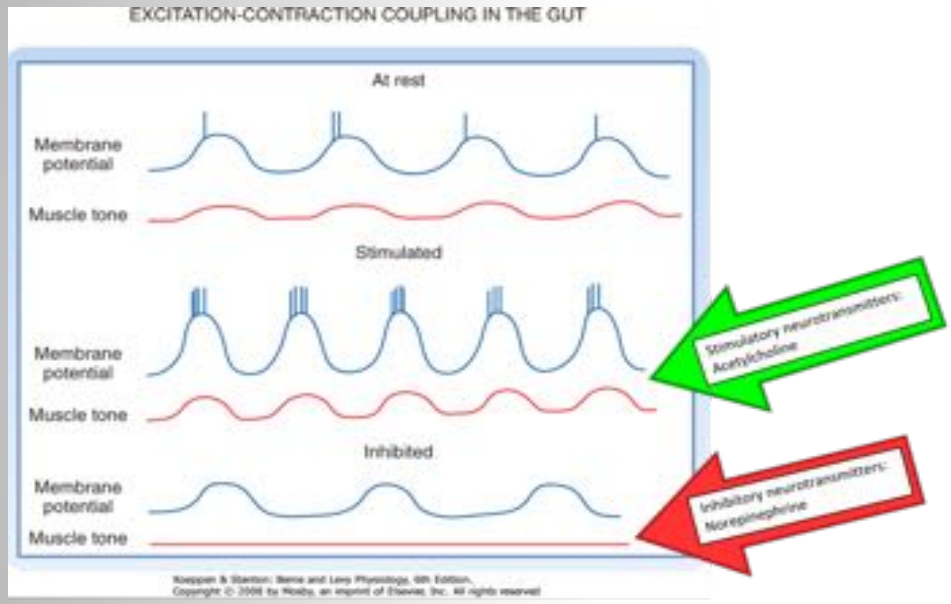

- Slow waves consist of an upstroke depolarization followed by a plateau of depolarization which upon reaching the smooth muscle, "primes" a region of interest and once the local environment allows for the smooth muscle to generate a spike potential in which threshold is reached, an action potential is triggered, allowing the normal mechanism for smooth muscle contraction to take place aka mechanism of peristalsis.
- Sympathetic neurotransmitters (i.e. NE) interact locally with the smooth muscle cell and can decrease the amplitude of the plateau depolarization phase of slow wave transmission below threshold, limiting the number of spike potentials that can be triggered while parasympathetic neurotransmitters (i.e. Ach) can cause an excitatory effect on the amplitude.

- **Mice with heart failure have decrease in amplitude with no change in frequency or damage in ICC networks.**
- ***We theorize that as a consequence of vascular calcification induced heart failure, the body reacts with sympathetic overactivity and release of sympathetic neurotransmitters, inducing an inhibitory effect on the amplitude of slow waves and indirectly decreasing GI smooth muscle contraction, causing gastric dysmotility.***
- **Further investigation should be done to assess levels of excitatory or inhibitory neurotransmitters as well as activity of the ANS in these mice groups to provide more evidence for our theory. Future studies on the effect of the ANS on the mechanisms of slow wave transmission may be a potential area of interest for drug therapy in gastric dysmotility disorders**
- **Establishing this connection may allow for future studies on the effectiveness of osteopathic techniques, such as rib raising which can help regulate the sympathetic chain, on patients with GI complaints that have HF.**

Acknowledgment

We would like to thank Mohnish Singh for echocardiograph analysis, Dr. Farajidavar and our colleagues at the Engineering Department NYIT for their work on analysis of slow waves, Dr. Randy Stout for confocal microscopy, Shadia Ahmed for technical support and the Makers of Dragonfly

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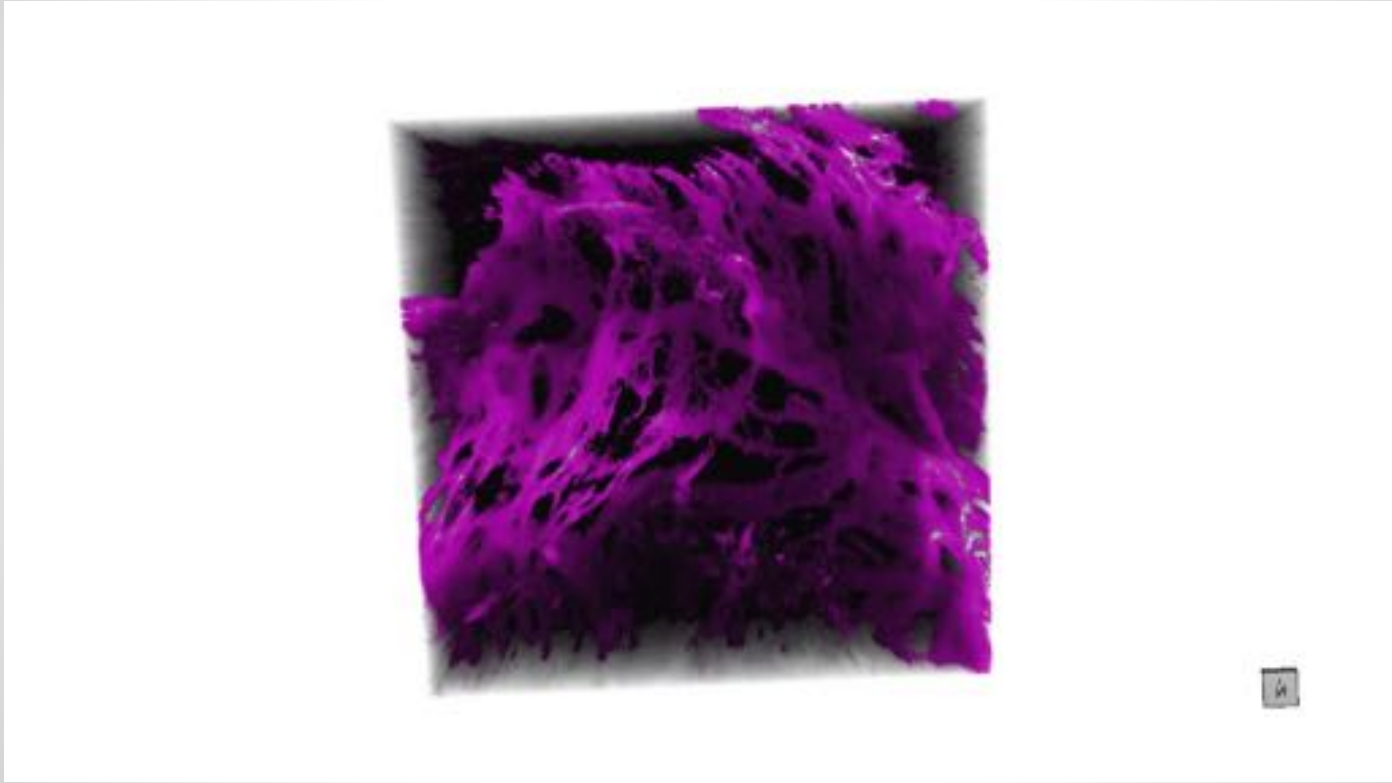

Back to Method

3D ICC Network was obtained through Confocal Microscopy