Stress-Induced Signaling in Aortopathy

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BACKGROUND

- Chronic psychological stress has been associated with poor health outcomes and increased risk for developing cardiovascular disease and hypertension.
- Patients with post-traumatic stress disorder (PTSD), a disorder of extreme stress, are known to have higher basal heart rates, blood pressure, and circulating proinflammatory cytokines and blood cell counts (white, red, and platelets).
- In vascular pathology, mechanical stimuli trigger a phenotypic switch in quiescent fibroblasts resulting in maladaptive myofibroblasts that cause disorganized extracellular matrix remodeling.

MOUSE MODELS

Generalized Stress

- Spontaneously hypertensive mice (BPH/2J) were created from an 8-way cross selecting for mice with elevated systolic blood pressures. Their control normotensive counterparts (BPN/3J) were randomly mated.
- BPH/2J mice develop hypertension by 5-weeks of age that is neurogenically derived and independent of the renin-angiotensin system.
- It has been shown that BPH/2J mice have an anxiety-like phenotype with alterations in key brain regions such as the medial amygdala, the paraventricular nucleus, and the rostral ventrolateral medulla.

PTSD

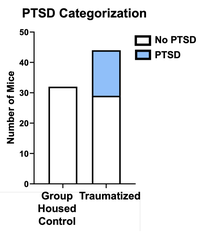
Induction Paradigm

- Inescapable Foot Shock (5 x 1 second foot shocks at 1.0 mA over 6 minutes preceded by a 20 second, 80 db, 9 kHz tone (conditioned stimulus)
- Single Prolonged Stress Events (2-hour Prolonged restraint, 20-minute group swim, loss of consciousness induced by anesthesia)
- Single housing following PTSD induction

Quantification of PTSD-Like Phenotype

- 4-weeks after induction, mice undergo a battery of behavioral tests chosen to assess for each of the human criteria of PTSD in the DSM-V (Table 1)
- Z-scores are generated for each behavioral test and mice with z-scores outside of the 85% confidence interval on all 5 criteria are defined as having PTSD (Table 1)

Criteria	Symptoms	Behavioral Assessment	Point
B	Intrusive Thoughts	Trauma Specific Reminder	1
C	Avoidance of Stimuli	Elevated Plus Maze & Light/Dark Box	1
D	Negative Alterations in Cognition	Barnes Maze & Sucrose Preference	1
E	Alterations in Arousal and Reactivity	Barnes Maze, Elevated Plus Maze, Light/Dark Box	1
G	Social Impairment	Crawley's Sociability Assay	1
	Non-Responders < 5	PTSD = 5	

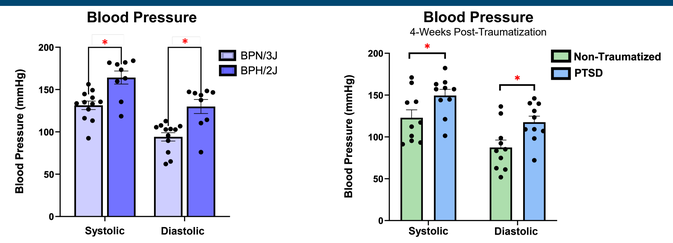


About 30% of traumatized mice developed a PTSD-like phenotype similar to incidence observed in humans exposed to trauma

HYPOTHESIS

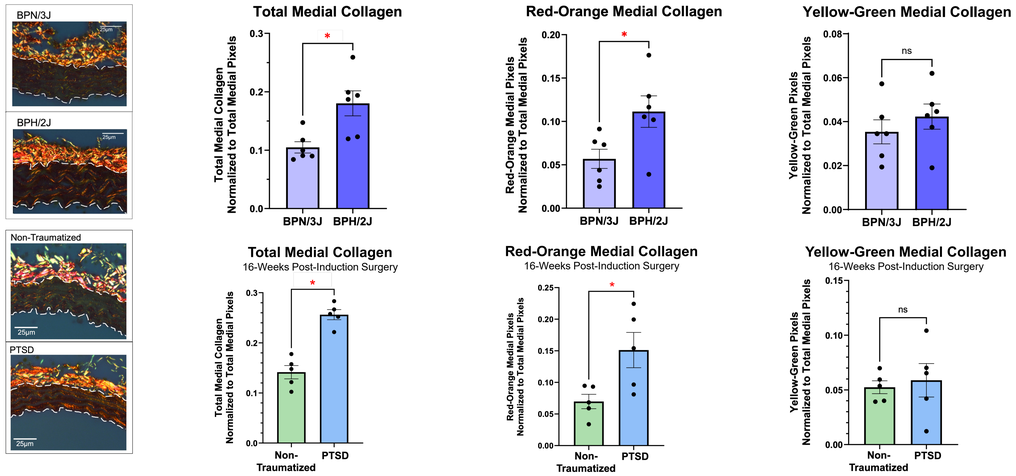
Mechanical signaling from psychological stress triggers extracellular matrix remodeling, which in turn can accelerate thoracic aortic aneurysm progression.

STRESS-INDUCED HYPERTENSION



Blood pressure was measured by tail cuff (Coda, Kent Scientific). Systolic and diastolic blood pressures were significantly elevated in **A)** BPH/2J mice over the BPN/3J mice (BPH/2J: n= 9, BPN/3J: n=12, p-value < 0.05) and **B)** PTSD-like mice over non-traumatized control mice (PTSD-like: n=10, Control: n=10, p<0.05)

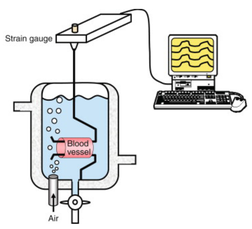
ENHANCED EXTRACELLULAR MATRIX REMODELING IN THORACIC AORTIC WALL

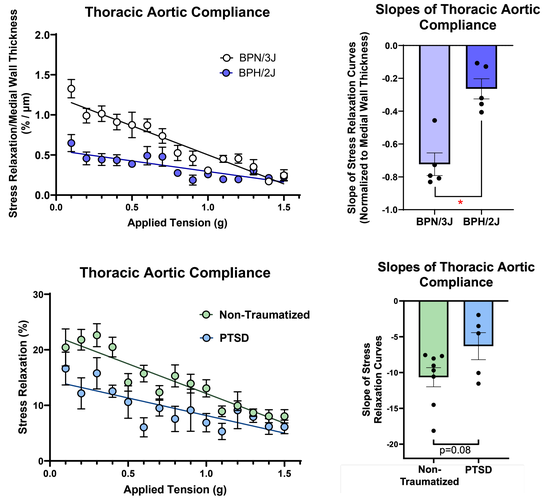


Thoracic aortas were analyzed histologically by picrosirius red staining under birefringence polarized light. Four different images were taken from two different thoracic aortic sections from each mouse. Red/orange cross-linked collagen and total collagen (red/orange and yellow/green fibrillar collagen) were elevated in the tunica media as determined by the number of red/orange and yellow/green pixels normalized to total number of pixels in the tunica media. (BPH/2J:n=6, BPN/3J: n=6, PTSD-like: n=5, Control: n=5, p-value < 0.05)

COMPENSATORY STIFFENING OF THE THORACIC AORTA

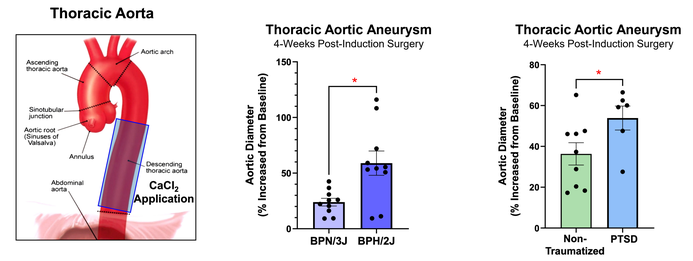
Parallel-Wire Myography





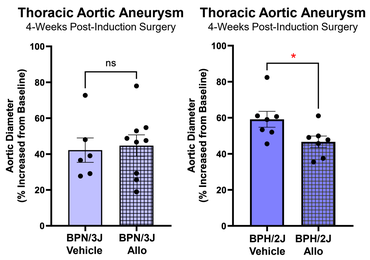
Descending thoracic aortas were mounted on parallel wires. Following a 30-minute equilibration, tension was applied to the parallel-wires in 0.1g increments from 0.1g to 1.5g. Thoracic aortas were allowed to relax for 3 minutes between increments of applied tension. Stress relaxation was determined from the difference in the starting tension and the residual tension calculated as a **B)** percentage or **A)** percentage normalized to the average medial wall thickness for each strain. BPH/2J and PTSD-like mice displayed a decrease in stress relaxation demonstrating a loss in thoracic aortic compliance. (BPN/3J: n=6, BPH/2J: n=6,PTSD-like: n=5, Control: n=8, difference in slopes p-value <0.05)

ACCELERATION OF THORACIC AORTIC ANUERYSMS



Thoracic aortic aneurysm (TAA) was induced by periadventitial application of 0.5M $CaCl_2$ to the descending thoracic aorta. This model results in reproducible dilatation and extracellular matrix remodeling of the treated aortic segment reaching a diameter greater than 1.5x its normal size over a 16-week period. Thoracic aortic diameter in BPH/2J and PTSD-like mice 4-weeks post-TAA induction surgery was shown to be larger than BPN/3J or control TAA mice demonstrating an acceleration of thoracic aortic aneurysm. (BPN/3J: n=10, BPH/2J: n= 10, PTSD-like: n=6, Control: n=9, * p<0.05). Thoracic aortic diameter for each animal was measured at baseline and terminal surgery by digital microscopy with the PaxCam software.

SUPPRESSION OF THORACIC AORTIC ANEURYSM ACCELERATION



Allopregnanolone (Allo), an endogenous neurosteroid that acts as a positive allosteric modulator of the $GABA_A$ receptor, has been implicated in the regulation of neurogenic hypertension and treatment of neuropsychiatric symptoms. Mice were pretreated with Allo (5mg/kg/day, mini-osmotic pump) or vehicle (50% w/v Captisol) for 2-weeks. Following the pretreatment, mice underwent surgical thoracic aortic aneurysm induction with $CaCl_2$ and their pumps were replaced with a 4-week mini osmotic pump delivering Allo (5mg/kg/day) or vehicle (50% w/v Captisol). Treatment with Allo decreased thoracic aortic diameter in BPH/2J 4-weeks post-induction surgery. There was no effect on thoracic aortic diameter in the BPN/3J mice. (BPN/3J: n= 6-9, BPH/2J: n= 7, * p-value < 0.05)

SUMMARY

- Stress-induced hypertension can alter thoracic aortic wall structure leading to elevated total medial collagen and enhanced collagen cross-linking all of which stiffen the thoracic aorta
- Elevated mechanical tension (neurogenic hypertension) can accelerate thoracic aortic aneurysm progression
- Allopregnanolone may suppress neurogenic hypertension and thoracic aortic aneurysm progression

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