Changes in MicroRNA Expression in Range Safety Officers Following Repetitive Sub-concussive Blast Exposure

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Background

- The majority of blast injuries in military service members occur during training
- DoW Safety Guideline Threshold: 4 Psi limit
- Physiological effect of training related blast exposure poorly understood

THE INVESTIGATING TRAINING ASSOCIATED BLAST PATHOLOGY STUDY

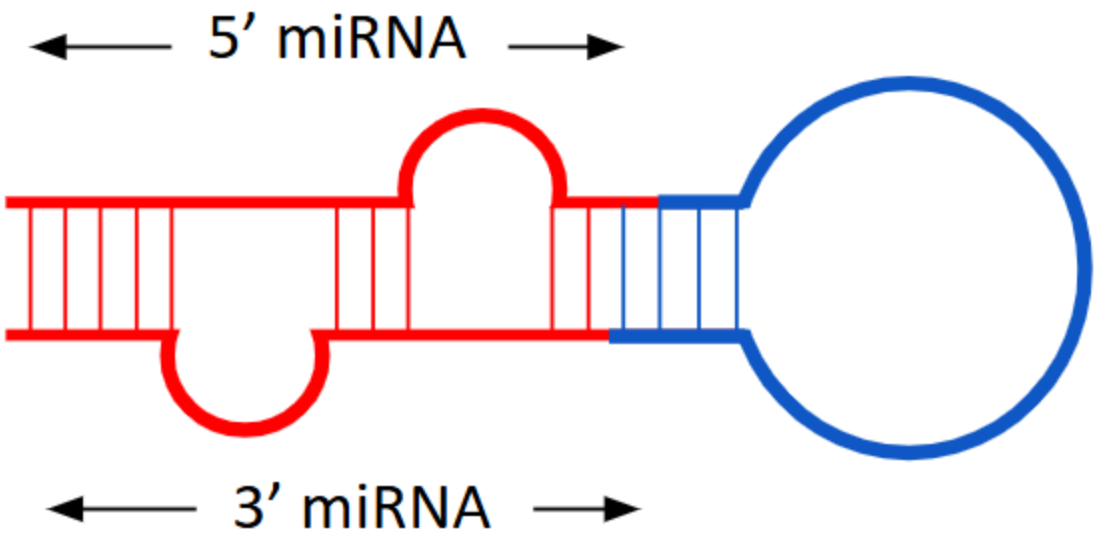
INVICTA STUDY

5-year prospective controlled longitudinal study of the impact of repetitive sub-concussive blast exposure (RSCBE) during Heavy Weapons Training (HWT)

Need for objective biomarkers for blast exposure in order to understand the effects of blast on the body

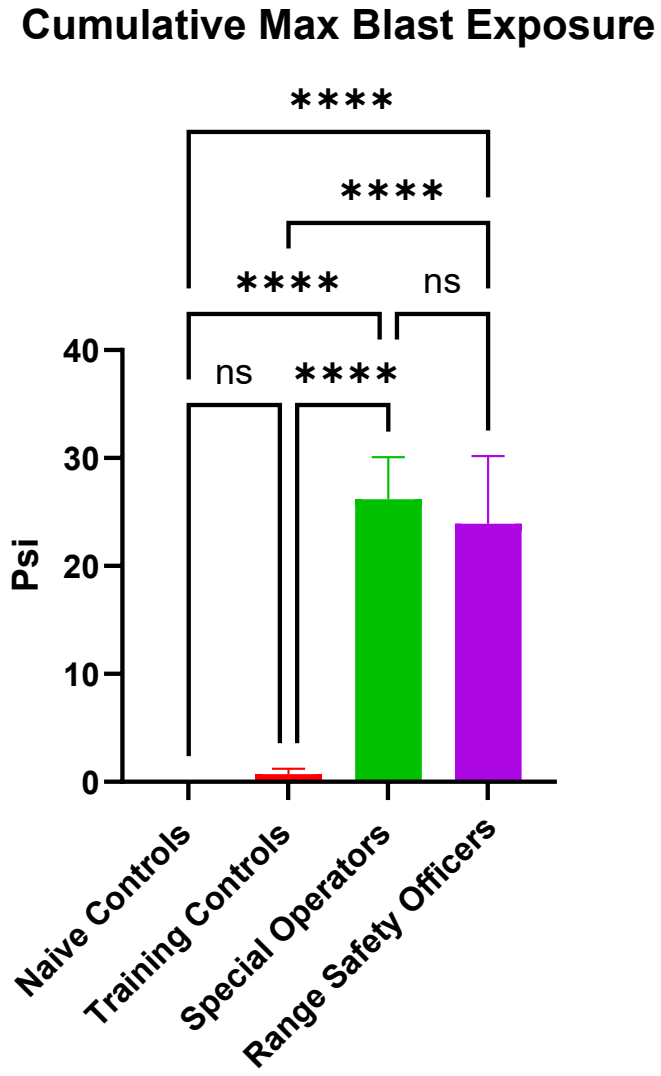
MicroRNA (MiRNA)

- Small non-coding RNA that regulate expression at the post-transcriptional level
- Key regulators of CNS function and potential biomarkers for injury
- Stability *in vivo* and *in vitro*
- Highly sensitive detection



Participant Demographics

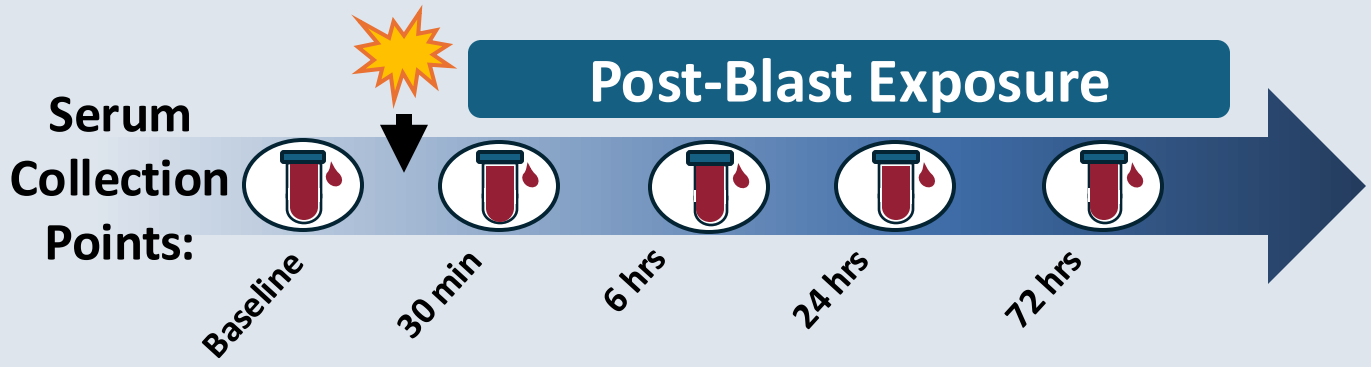
		Range Safety Officer (RSO)		Special Operator (SO)		Naive Controls (NC)		Training Controls (TC)	
n		31		60		21		54	
Race	White	29		54		16		47	
	Black or African American	0		1		3		1	
	Two or More Races	2		2		2		1	
	Asian	0		0		0		1	
	Hispanic	0		1		0		1	
	Native Hawaiian or Other Pacific Islander	0		1		0		0	
	N/A	0		1		0		3	
		Average	SD	Average	SD	Average	SD	Average	SD
Years of Service		9.23	3.24	5.80	2.78	13.48	5.63	4.77	5.05
Age at Baseline		31.07	3.69	28.17	3.15	32.10	9.21	27.09	4.73
# of Heavy Weapons Trainings		6.03	5.32	2.30	1.29	4.50	3.32	3.79	5.84
Times Deployed		3.00	1.26	1.82	1.07	3.83	2.46	2.85	2.39
Total Months of Deployments		18.48	8.17	10.10	6.54	27.11	2.66	15.70	10.77
# of Combat Deployments		1.83	0.82	1.35	0.67	3.25	0.96	2.73	2.05



**** = P < 0.0001

Methodology

Timeline & Sample Collection



Two-Phase Experimental Pipeline

Phase 1: Initial miRNA Screening

Discovery Set (n=94)
OpenArray panel targeting 224 miRNAs

Phase 2: ddPCR Validation

2 Differentially Expressed miRNAs validated across the full cohort (n=166)

OpenArray Analysis of NC and TC compared to RSOs revealed miRNAs implicated in mechanically-included TBI.

		Baseline	30 min	6 hours	24 hours	72 hours
miR-221-3p	**NC v. RSO**	0.7180	0.0868	0.0515	0.0004	0.00004
	TC v. RSO	**0.0444**	0.8463	0.3636	**0.0004**	**0.0017**
miR-338-3p	**NC v. RSO**	-	**0.0259**	0.08	-	0.2090
	TC v. RSO	0.7567	0.7711	0.3636	**0.0009**	**0.0023**

Functional Roles of MiR-338 and MiR-221

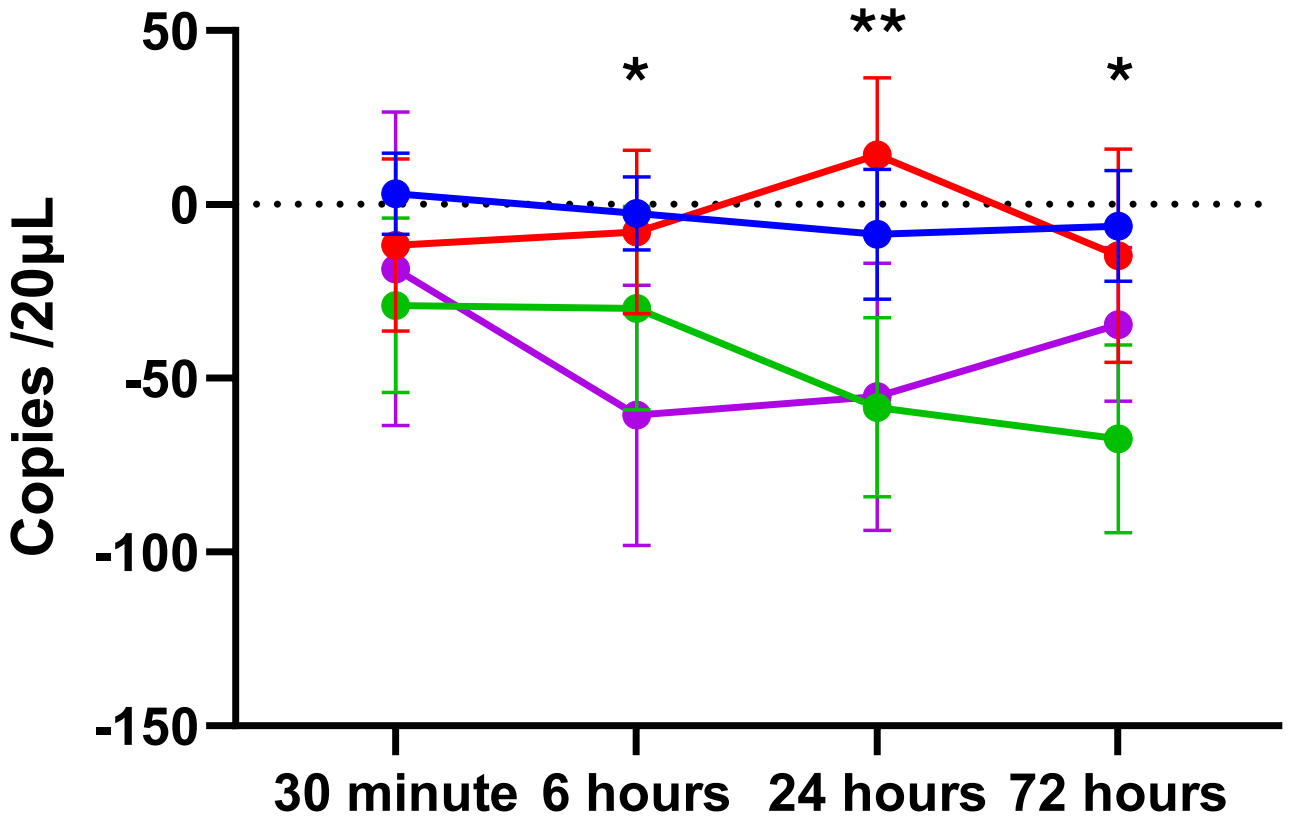
MiR-338

- Neuronal Maturation
- Axonal guidance
- Mitochondrial activity and function
- Neurogenesis
- Oligodendrocyte differentiation

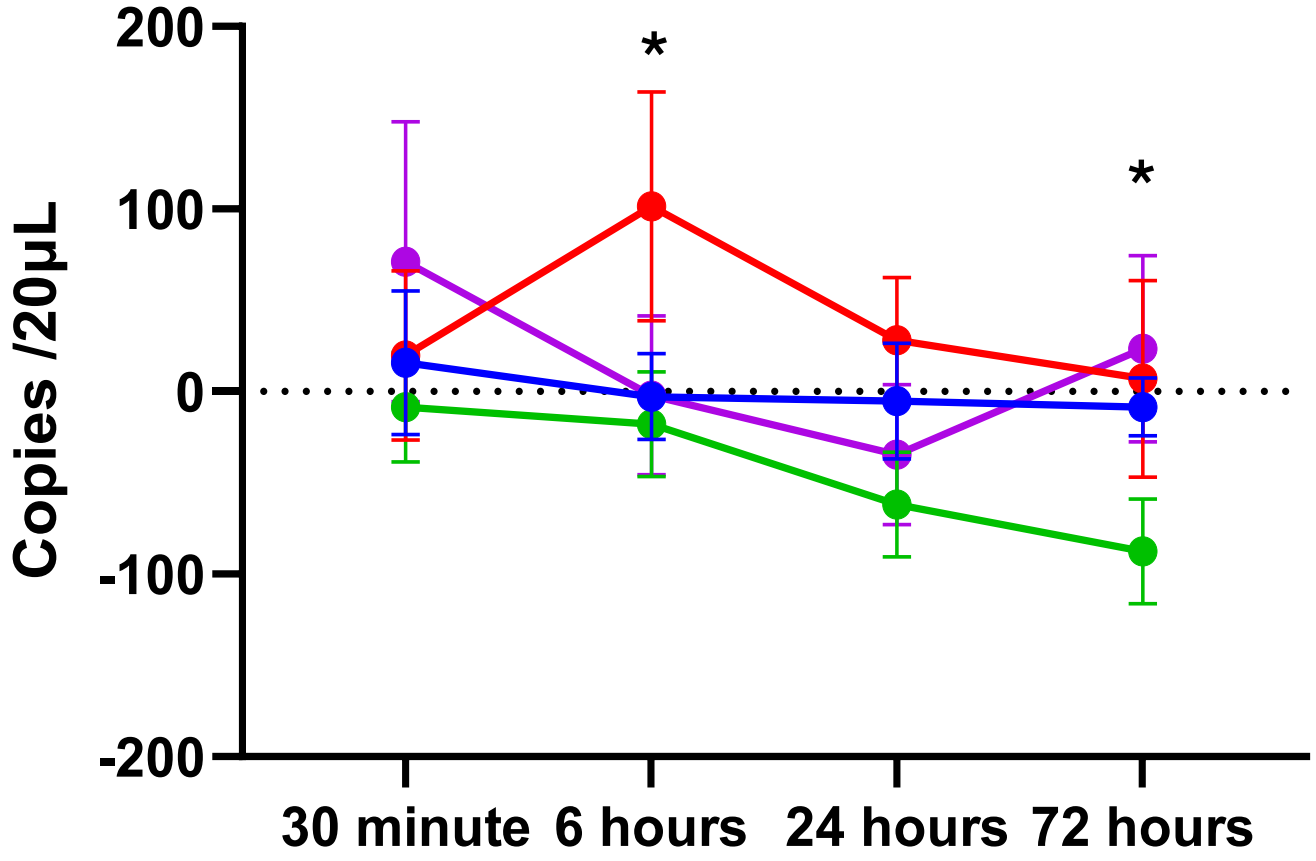
MiR-221

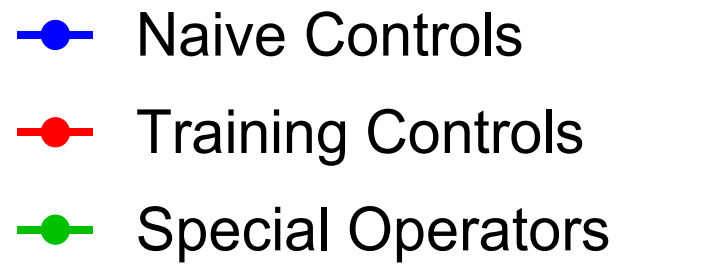
- Neuronal Survival and proliferation
- Nerve Regeneration
- Suppression of Neuroinflammation
- Protection against Neurotoxicity

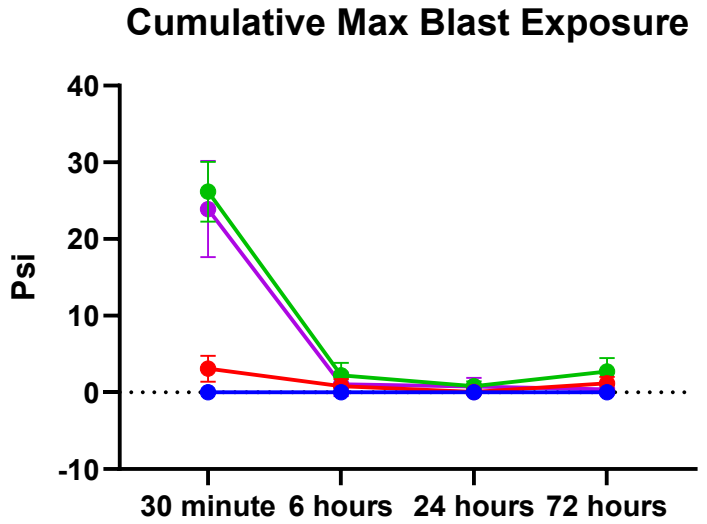
DDPCR analysis of MiR-338-3p revealed a significant decrease following blast exposure in RSOs and SOs.



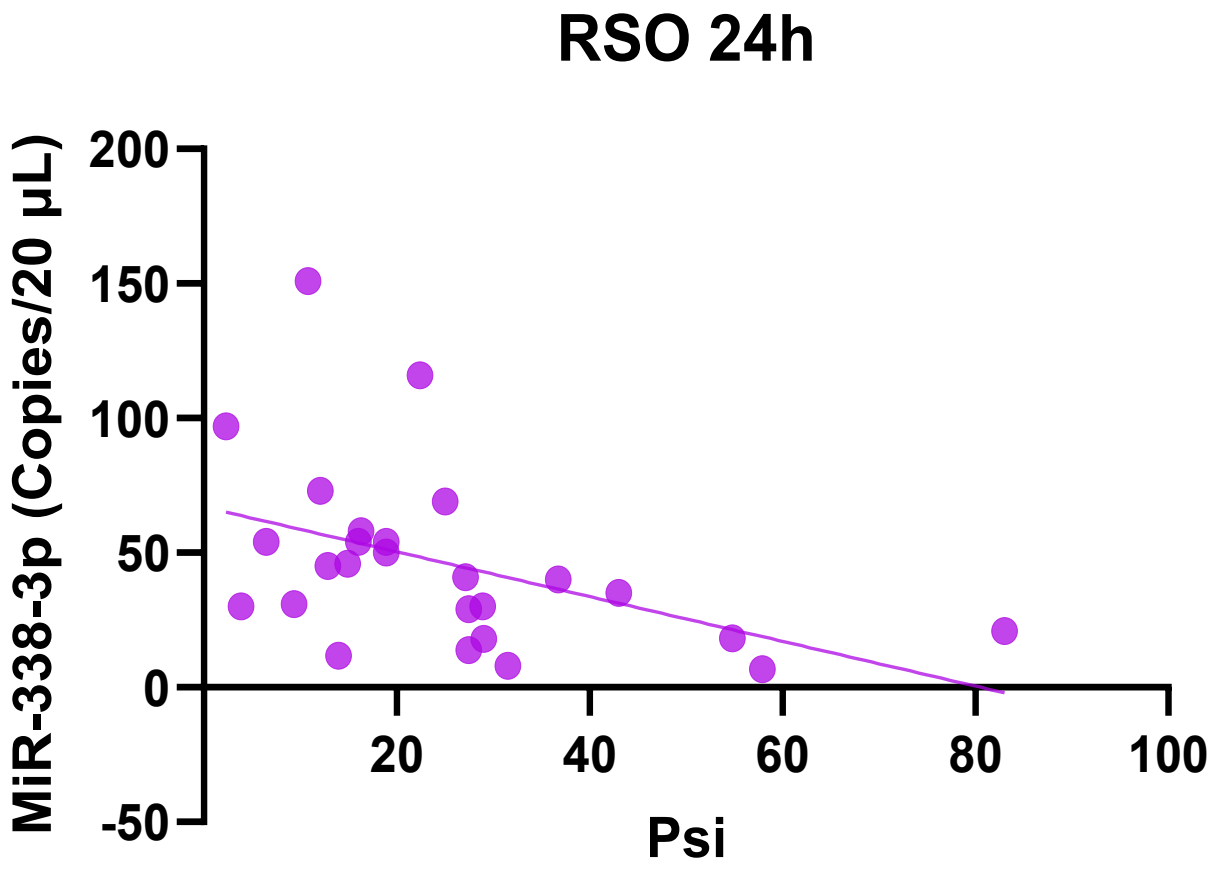
DDPCR analysis of MiR-221-3p revealed a significant decrease following blast exposure in RSOs and SOs.







MiR-338 concentration correlates with cumulative blast exposure in RSOs.



Significant negative correlation suggests that blast-induced loss of this miRNA could drive injury progression by impairing myelination in the CNS

R^2 = -0.569
P = 0.0024

Conclusions

- RSCBE alters circulating CNS-related miRNA
- miR-338-3p and miR-221-3p may serve as early markers for RSCBE
- **Downregulation may signal a failure of compensatory repair and cell survival mechanisms**
- Future studies will use machine learning and long-term outcomes to refine risk assessments for blast-exposed Service Members.

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