AFRL

EXPLOITING RADIO FREQUENCY ELECTROMAGNETIC FIELD HORMESIS TO PROVIDE RESILIENCE TO NEURONS

Ibtissam Echchgadda, PhD, Senior Research Biologist

711HPW/RHDR

The 2024 Human Performance and Biosystems Program Review, October 2024

The views expressed are those of the authors and do not reflect the official guidance or position of the United States Government, the Department of Defense, the United States Air Force or the United States Space Force. Unless otherwise noted, imagery in this document are property of the U.S. Air Force.

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, Sept 26 2024.

Hormetic or Adaptive Response (AR)

Low-Mild Stress

- ✓ Adaptive dose (AD)
- ✓ Conditioning Dose
- ✓ Hormetic Low-dose

Hormetic Response

Unfold protein response ⇑
Heat shock protein ⇑
Adaptive mtROS signaling ⇑
Autophagy ⇑
Epigenetic impri[illegible]

Preconditioned State

Stress resistance

Enhanced cellular defense response ⇒⇒ Cell survival

Severe Stress

- ✓ Challenging dose (CD)
  - ○ High dose of same or different stressor

No Stress

No preconditioning

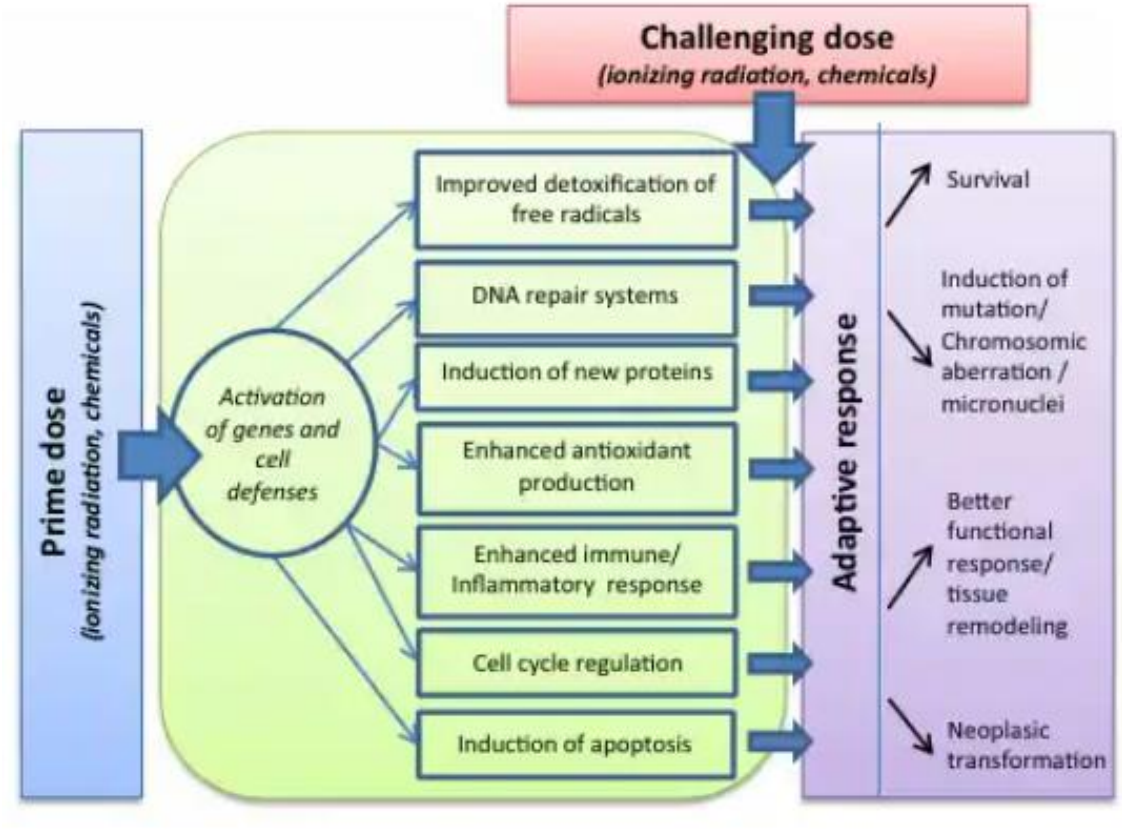
No Hormetic Response/AR

Cell Damage (Injury)

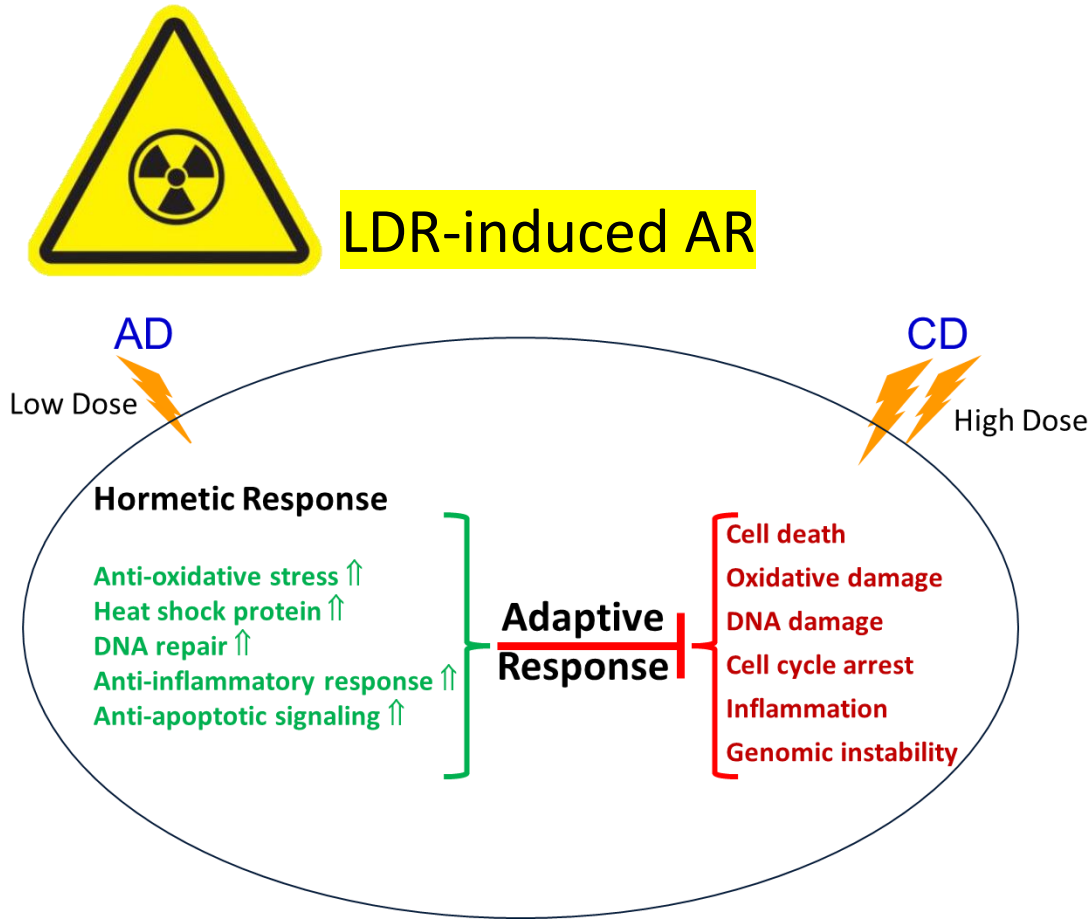
Weaker defense ⇒⇒ Cell death

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Low-dose ionizing radiation (LDR), AD, can induce AR and protection from CD of subsequenst stressors



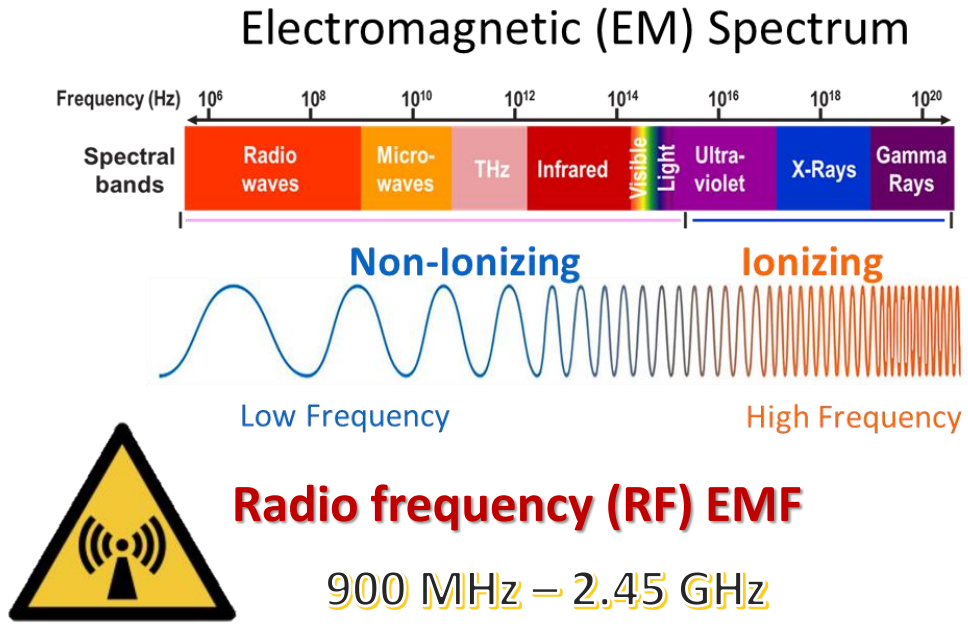
Guéguen Y et al. Adaptive responses to low doses of radiation or chemicals: their cellular and molecular mechanisms. Cellular and Molecular Life Sciences (2019).



Adapted from Jin S et al. New understanding of the low-dose radiation-induced hormesis. Radiation Medicine and Protection, (2020).

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Does non-ionizing EM fields (EMF) pre-exposure induce hormetic or adaptive response?



Radio frequency (RF) EMF

900 MHz – 2.45 GHz

There are a few report in literature of RF EMF (pulsed, modulated) preconditioning potential to induce hormetic or AR, resulting in protection from adverse effects from a subsequent stressors, both in cultured cells and in animal models.

- Remains to be confirmed by independent labs (⇒ dosimetry, temperature histories, dose response)
- Understand how/mechanisms RF-EMF pre-exposure (both continuous wave (CW), pulsed or modulated exposure modalities) would induce stress adaptation and cellular resilience
- Understand how to exploit/maximize the effect

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Project Goal and Hypothesis:

RF-EMF Exposure

(Mild Stress)

- Adaptive dose (AD)
- Conditioning dose
- Hormetic low-dose

Adaptive Response

Unfold protein response ⇑

Heat shock protein ⇑

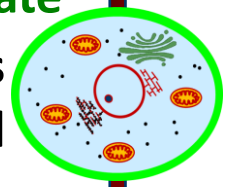
Adaptive mtROS signaling ⇑

Autophagy ⇑

Epigenetic imprinting

Preconditioned State

Stress Resistant Cell



Cell survival

Subsequent Insult

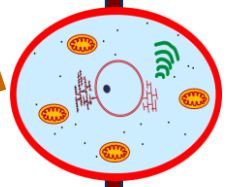
- Challenge dose (CD)
- High-dose

AF occupational environments

Sham Exposure

(No RF-EMF)

No preconditioning



Cell Damaged (Injured)

Cell death

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

PROJECT AIMS AND PROGRESS

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317. 26 Sept 2024.

Project Aims

1. Investigate RF-EMF hormetic dose-response phenomenon in cells

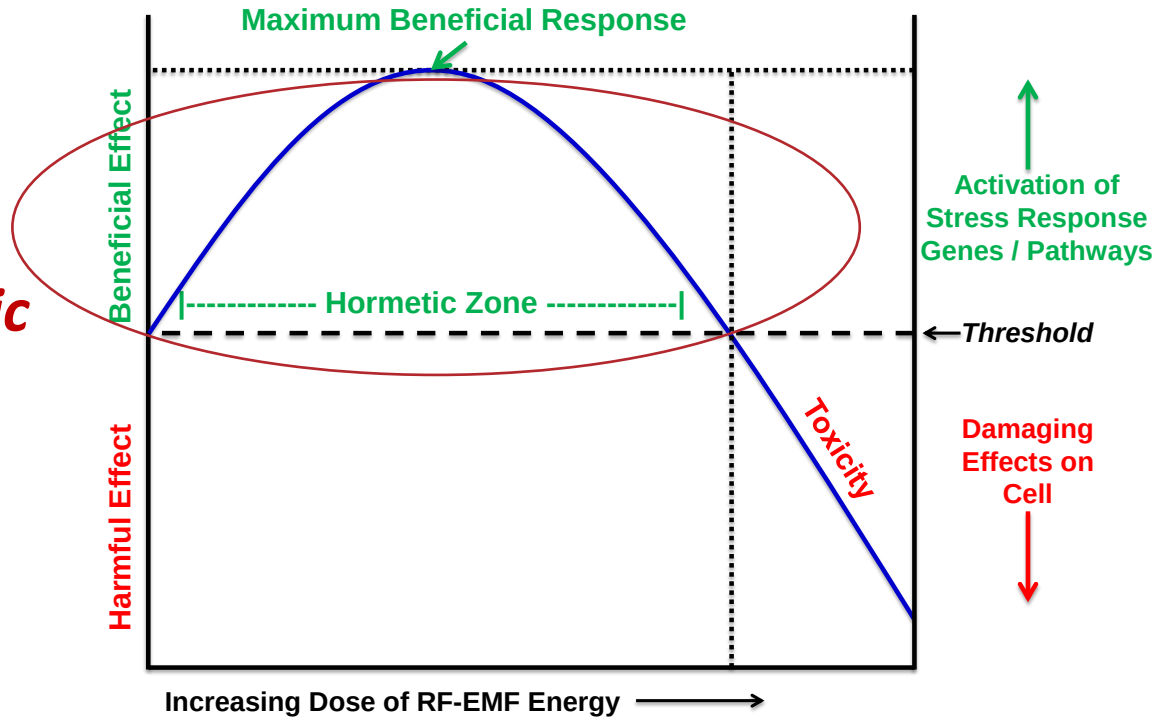
2. Assess if preconditioning with an adaptive dose (AD) of RF-EMFs would confer adaptive response (AR) and cell resilience to a subsequent exposure to stressors

3. Investigate additive/synergistic effects of RF-EMF in combination with selected preconditioning agents

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

1. Investigate RF-EMF hormetic dose-response phenomenon in cells

Can we establish RF-EMF hormesis (biphasic dose–response or Hormetic response curve) in cells?

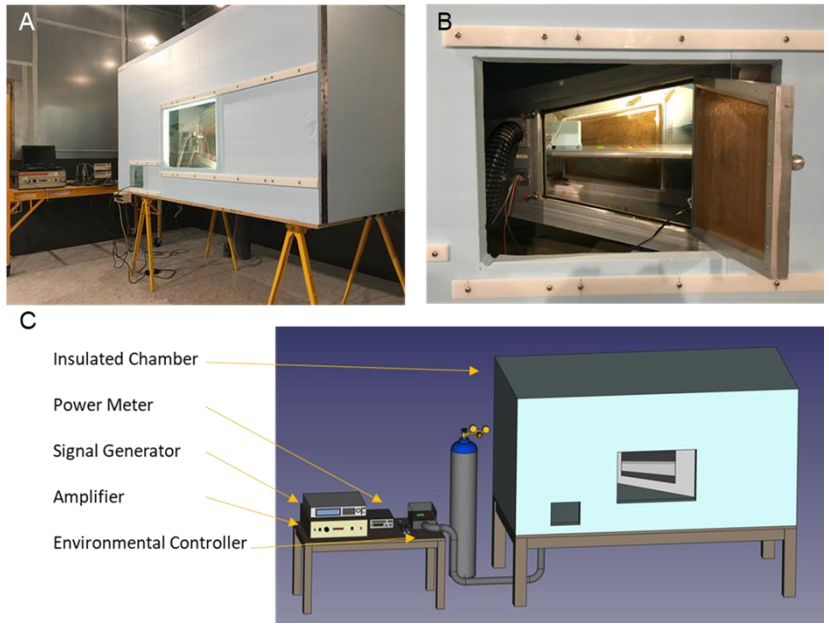


DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

1. Investigate RF-EMF hormetic dose-response phenomenon in cells

- Empirical exposures at different conditions: Frequencies and durations

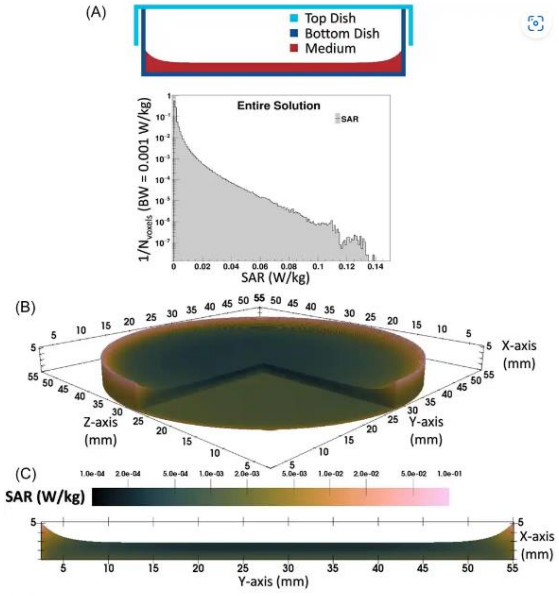
–**CW,** pulsed or modulated exposures



Custom, environment-controlled exposure system
(37 ºC, 5% CO_2, 95% RH)

- Dosimetry of RF-EMF Exposures

-Finite-difference time-domain (FDTD)



(D)

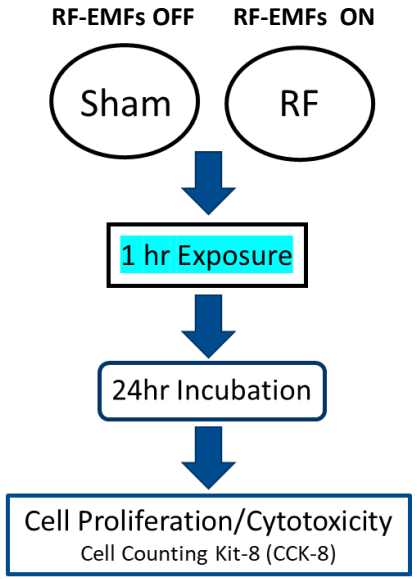
E-field (V/m)	Bottom Layer		Entire Solution	
	Average SAR (W/kg)	S.D. (W/kg)	Average SAR (W/kg)	S.D. (W/kg)
40	1.55×10^{-3}	7.04×10^{-4}	1.63×10^{-3}	3.11×10^{-3}

Cantu JC, Butterworth JW, Peralta XG, Payne JA, Echchgadda I. Bioelectromagnetics (2023). https://doi.org/10.1002/bem.22439

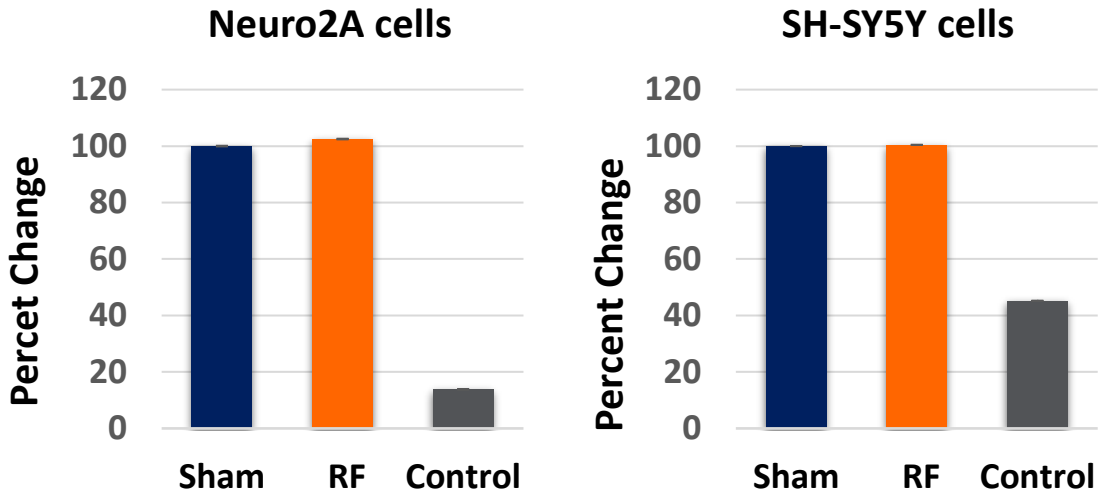
DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

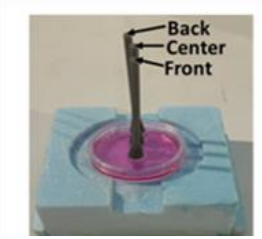
CW RF-EMF dose, duration, and timing of the hormetic effect

- Effect on cell proliferation/viability in neuronal cells



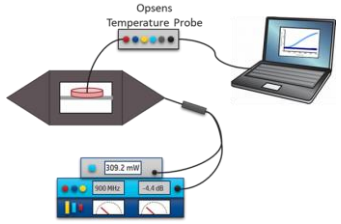
Representative Results





CW, 1.8 or 2.45 GHz for 1 hour (Exposure set a dose below thermal (heating) threshold of 39°C - 40°C)

Media temperature



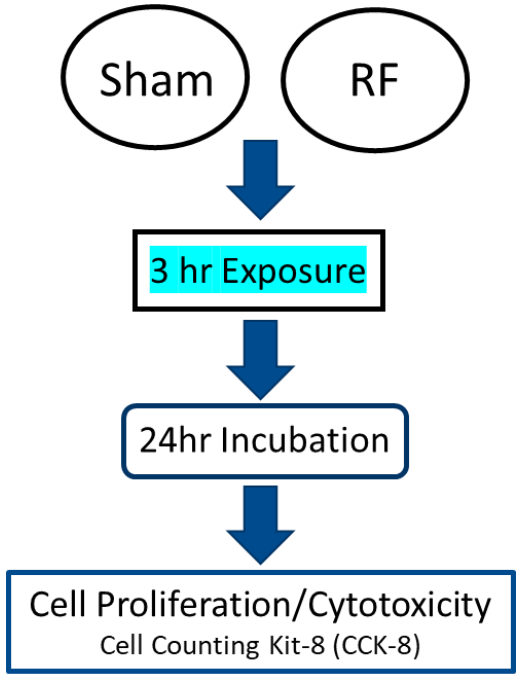
Media temperature

Temperature
Start Temp: 37°C
End Temp: 40°C

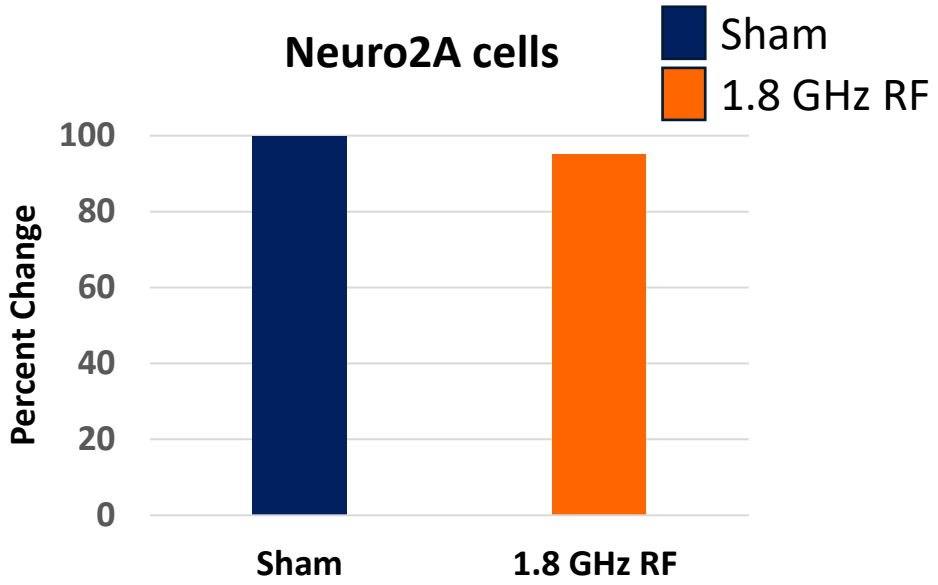
DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

CW RF-EMF dose, duration, and timing of the hormetic effect

- Effect on cell proliferation/viability in neuronal cells



Representative Results



CW, 1.8 or 2.45 GHz for 3 hours (Exposure set a dose below thermal (heating) threshold of 39°C - 40°C)

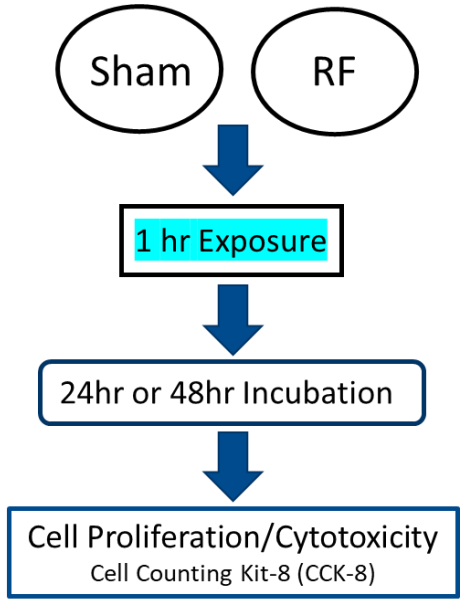
Media temperature

Temperature
Start Temp: 37°C
End Temp: 40°C

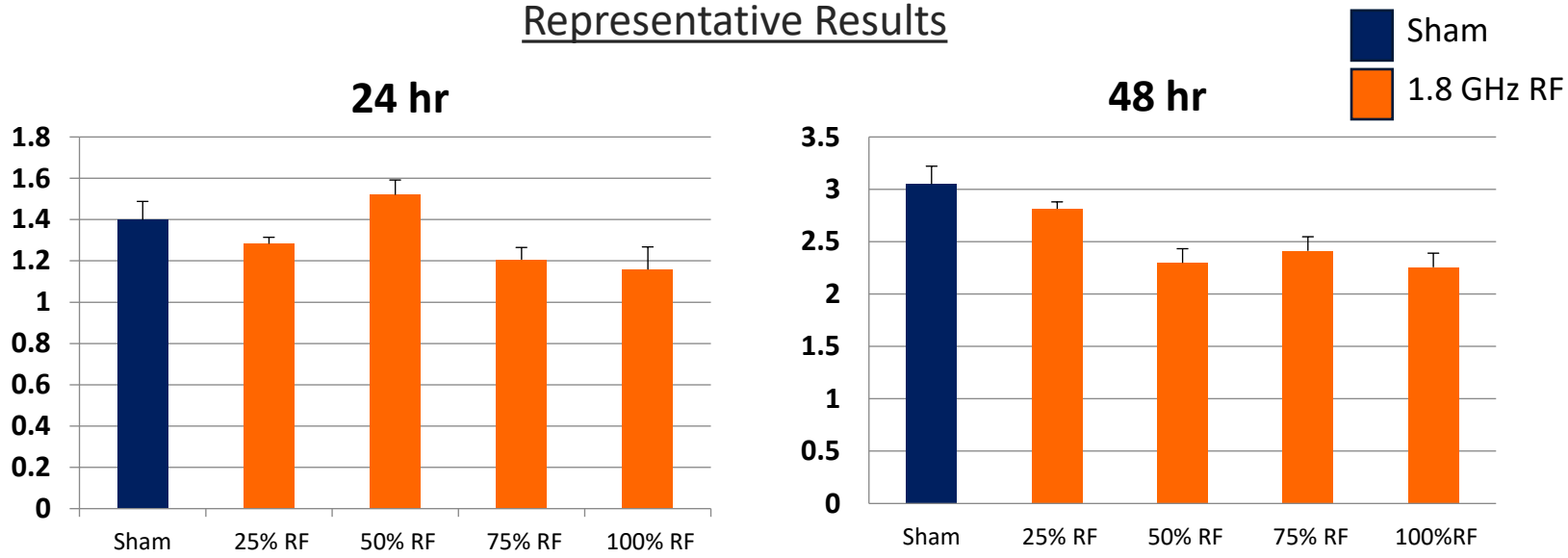
DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

CW RF-EMF dose, duration, and timing of the hormetic effect

- Effect on cell proliferation/viability in neuronal cells



Representative Results



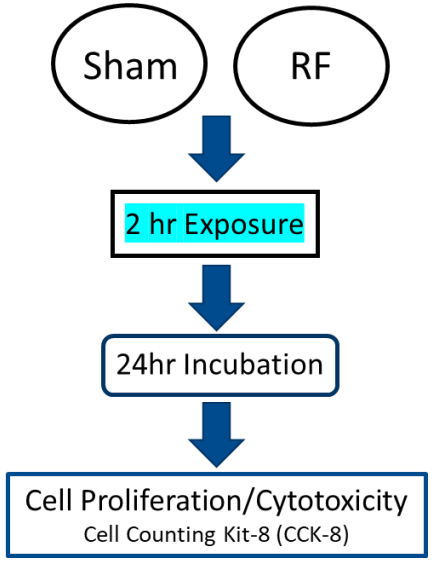
CW, 1.8 or 2.45 GHz RF exposure for 1 hour with decreasing doses from the initial dose (set below the thermal threshold of 39°C - 40°C).

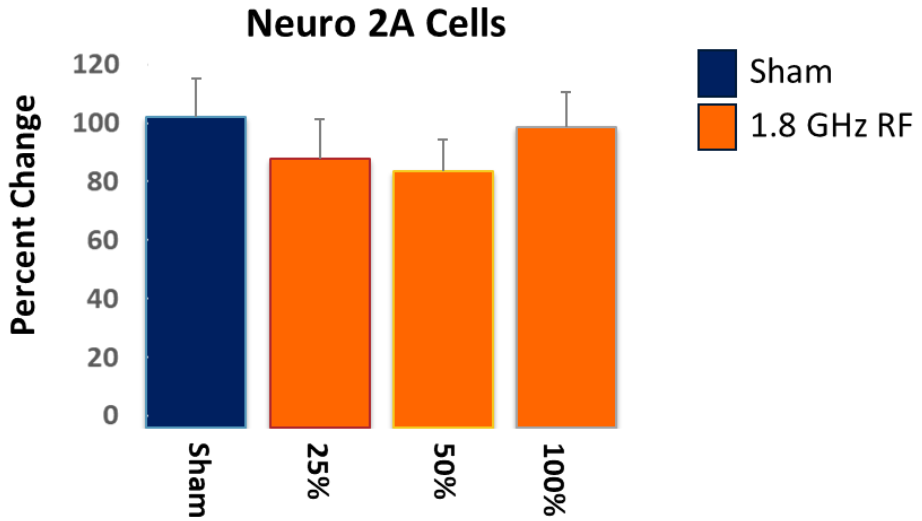
DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

CW RF-EMF dose, duration, and timing of the hormetic effect

- Effect on cell proliferation/viability in neuronal cells

Representative Results



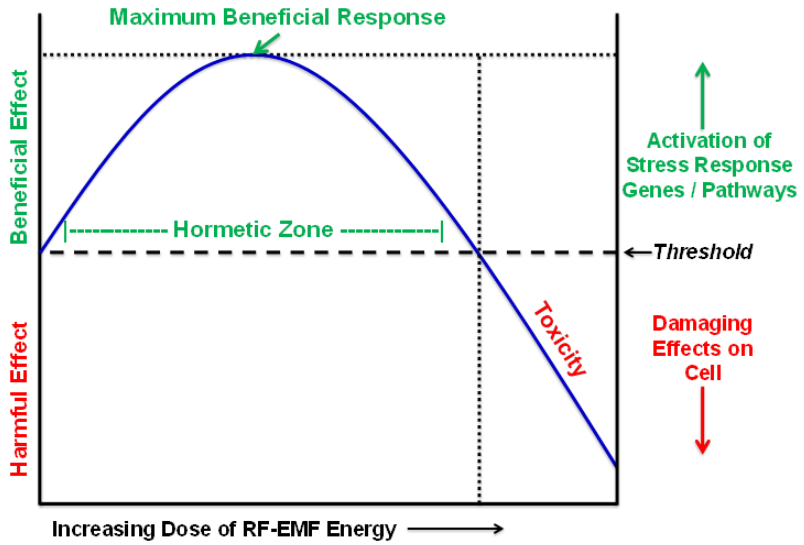


CW, 1.8 or 2.45 GHz RF exposure for 2 hours with decreasing doses from the initial dose (set below the thermal threshold of 39°C - 40°C).

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Project Aim 1 – Summary of Results and future directions

- 1. **Investigate RF-EMF hormetic dose-response phenomenon in cells**



- We focused on the effect of CW RF-EMF exposure on cell viability, assaying cellular response at single frequencies (1.8 GHz, 2.45 GHz), while varying only the signal intensity and duration.
- The results don't provide convincing evidence that a hormetic dose-response exist for CW RF-EMF exposures, assessing survival, in cells exposed to CW RF-EMFs under the conditions tested.

❑Future experiments:

- Continue testing effects of additional CW exposure conditions, and to include tests from pulsed RF-EMF exposures under similar SAR value doses.
  - Investigate the hormetic effects in presence of subsequent stress.
  - Assess additional end points, e.g., oxidative stress, autophagy, DNA damage, etc.

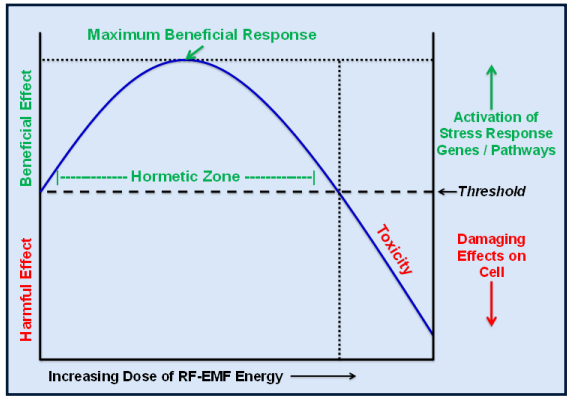
DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

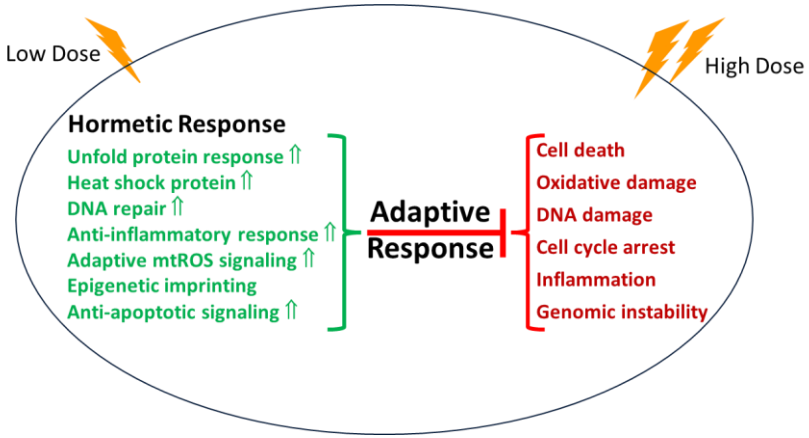
Project Aims

1. Investigate RF-EMF hormetic dose-response phenomenon in cells

2. **Assess if preconditioning with an adaptive dose (AD) of RF-EMFs would confer adaptive response (AR) and cell resilience to a subsequent exposure to stressors**

3. Investigate additive/synergistic effects of RF-EMF in combination with selected preconditioning agents

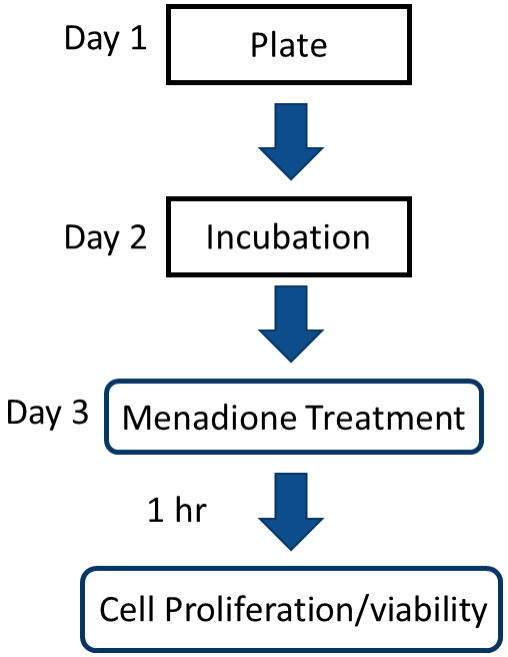




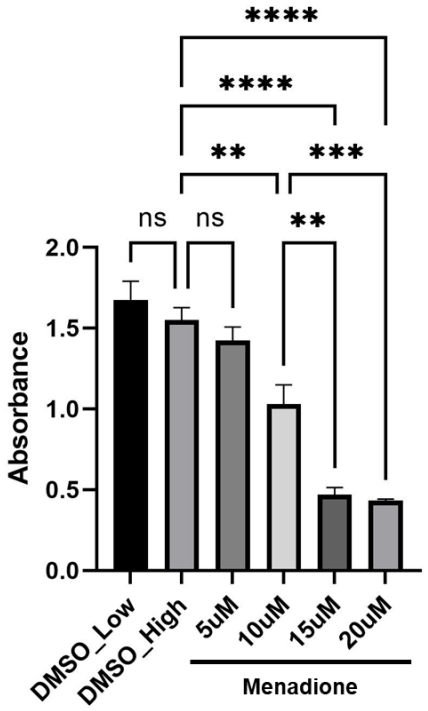
DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Assess if preconditioning (pre-exposure) with RF-EMFs would confer protection from to menadione-induced cell death

- Menadione Dose Response

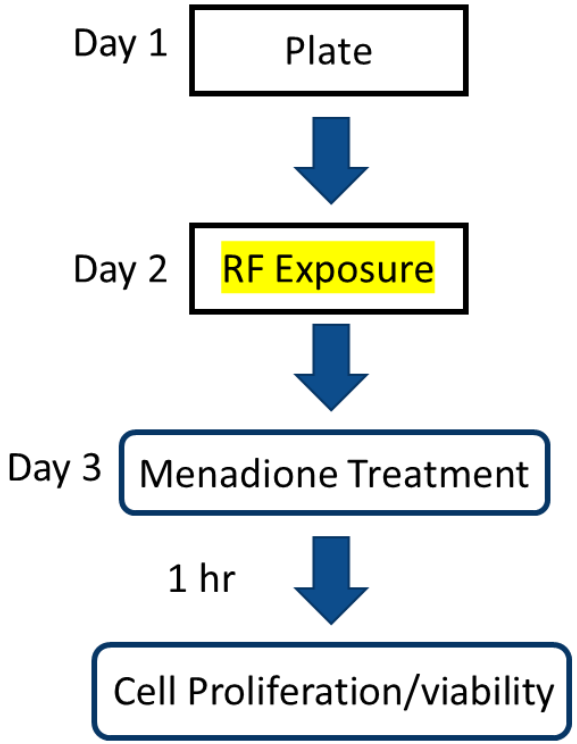


Effect on cell viability



*DMSO_Low= .005% DMSO
*DMSO_High= .02% DMSO

Preconditioning

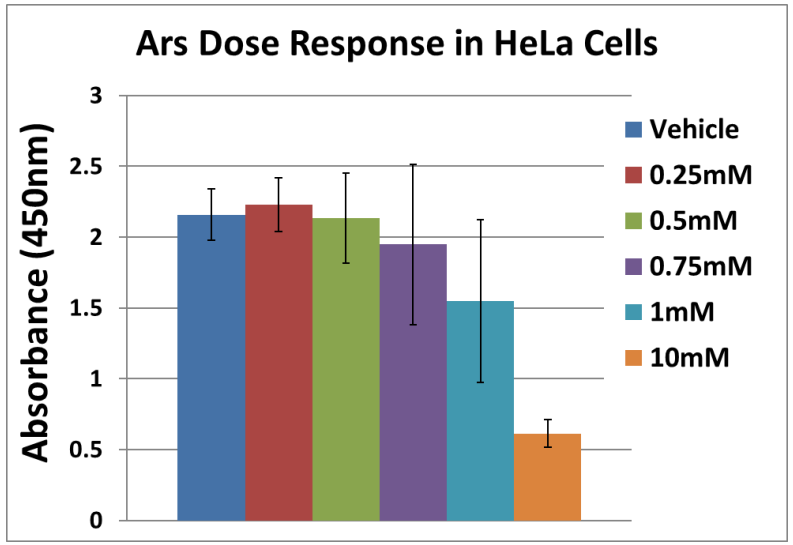


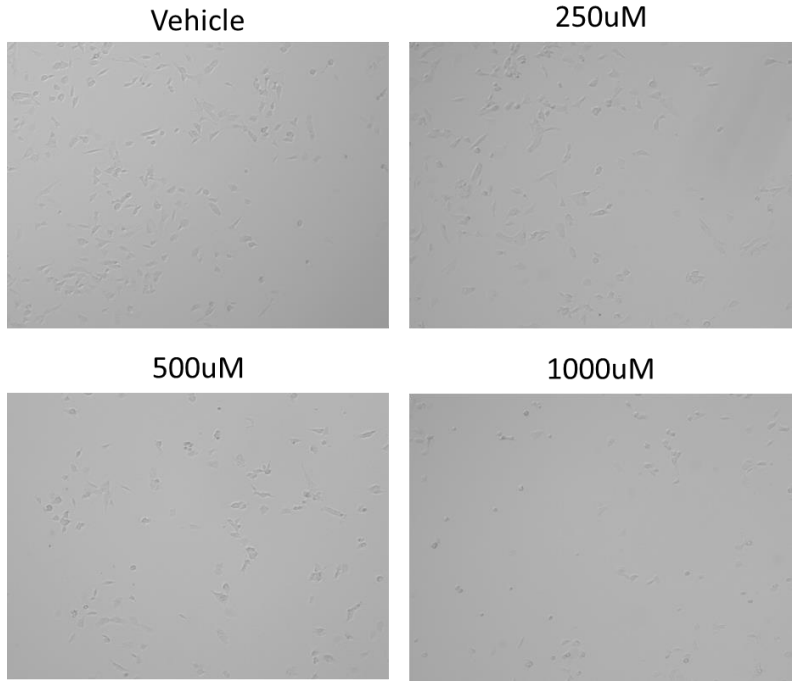
DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Assess if pre-exposure with RF-EMFs would affect sodium arsenite induced stress granules (SGs) and cell death

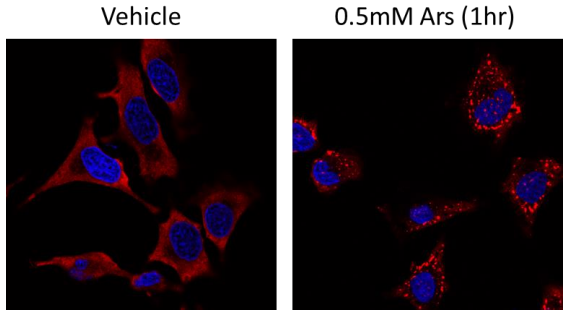
- Sodium Arsenite (Ars) Dose Response: Effect on cell viability and formation of stress granules

Cell viability





Stress Granules (G3BP1)

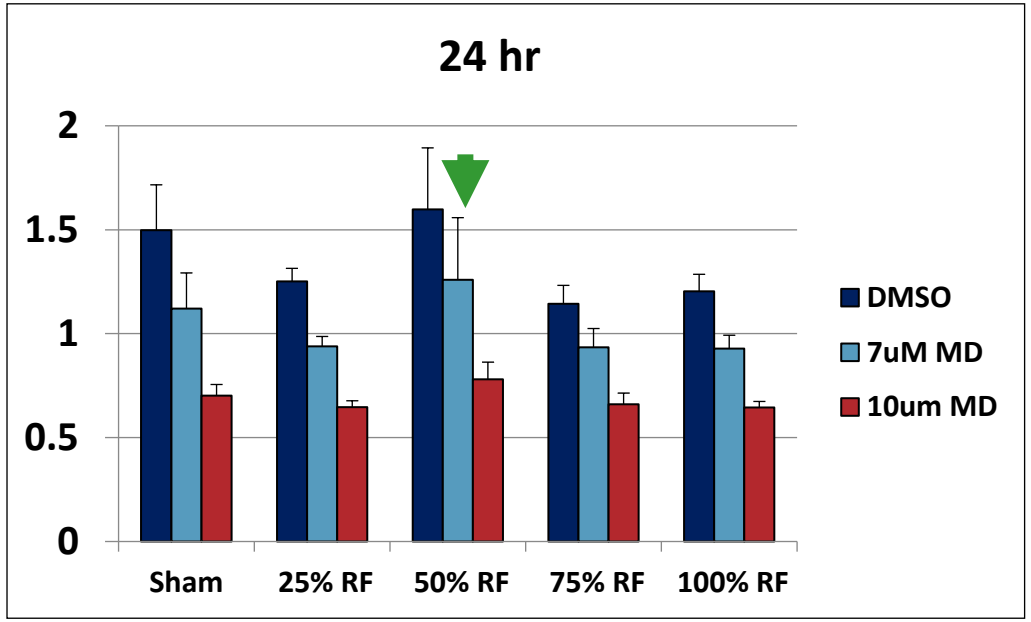


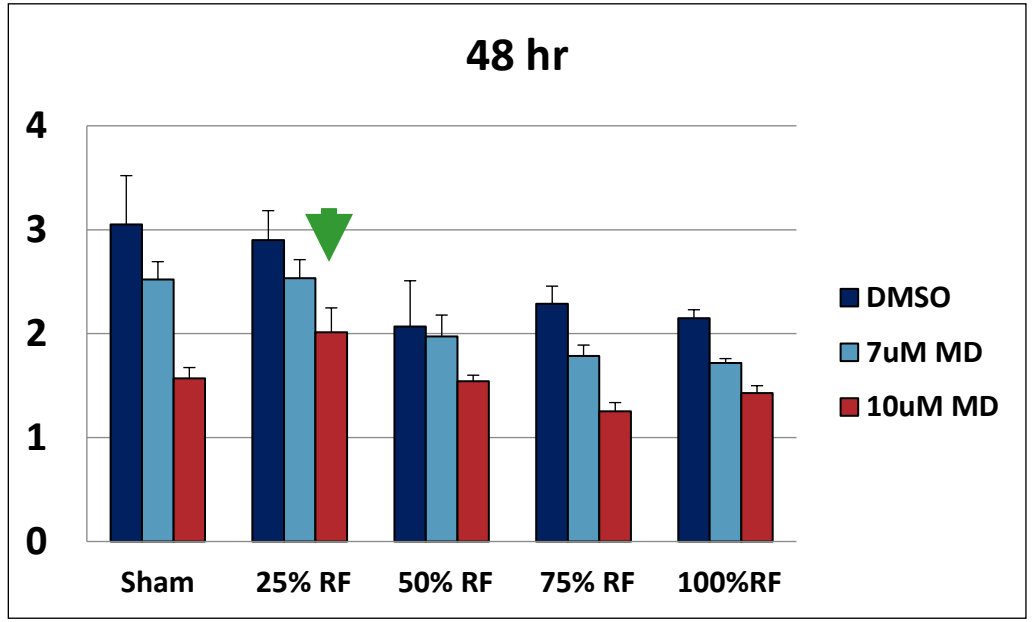
Ars induce SGs in Hela Cells. Ars or vehicle treatment for 1 hr. Immunocytochemistry (ICC) with G3BP1 (marker for SGs).

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Assess if preconditioning (pre-exposure) with RF-EMFs would confer protection from to menadione-induced cell death

- Effect on cell proliferation/viability in neuronal cells following menadione (MD) treatment

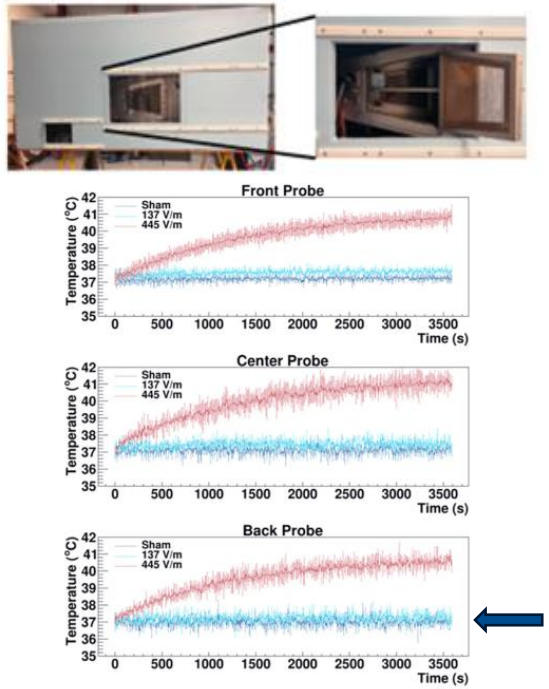




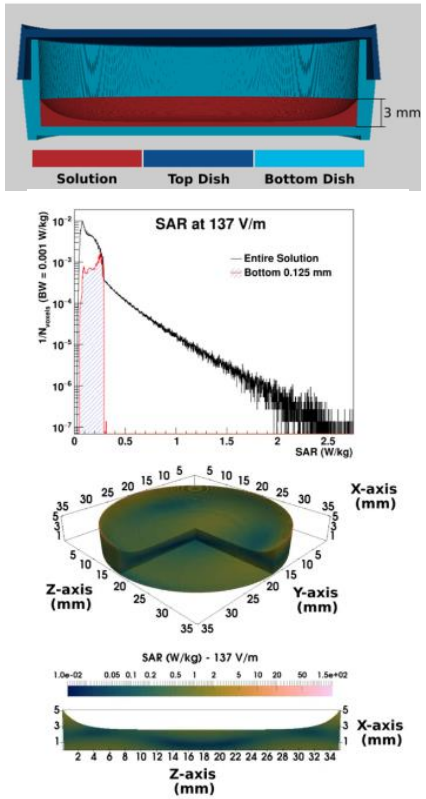
- There is a trend, but the results don't fully support an AR from exposure to CW RF-EMF under the conditions tested, investigating protection from menadione-induced cell death.
  - More data is needed

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Transcriptional response of primary hippocampal neurons following exposure to RF-EMFs (137 V/m for 1 hr)

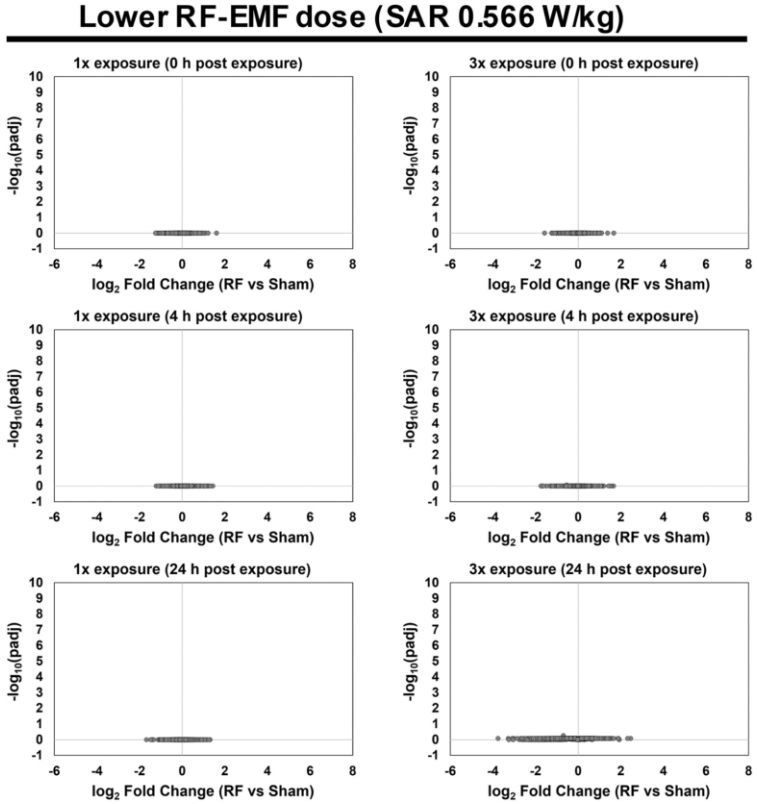


37°C - 38°C



SAR 0.566 W/kg

No differentially expressed genes identified with the low, non-thermal, RF-EMFs dose

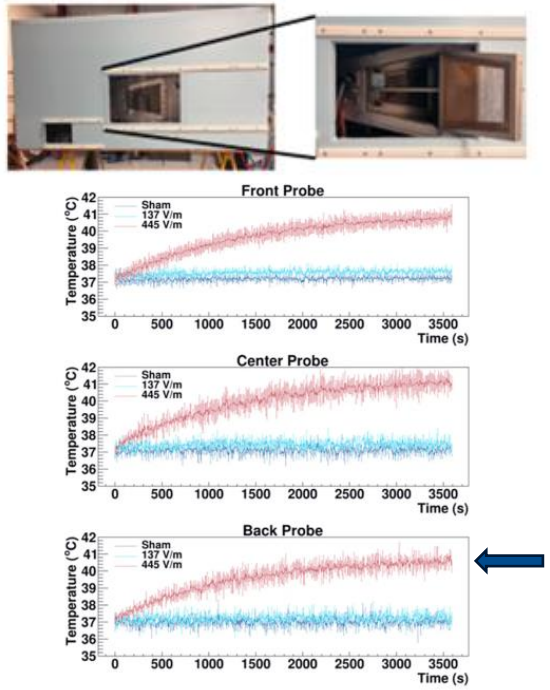


All genes did not meet the cutoff for significance set at FDR ≥ 0.05 (|FC|≤2).

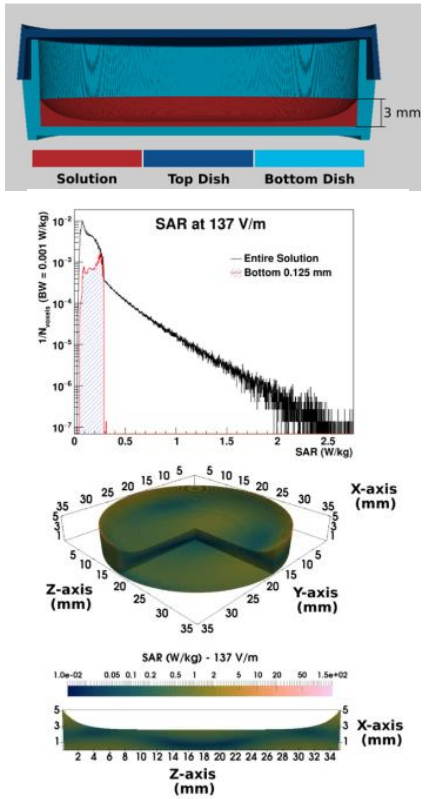
Cantu JC, Butterworth JW, Payne JA, Echchgadda I. Bioelectromagnetics (2024). https://doi.org/10.1002/bem.22517

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Transcriptional response of primary hippocampal neurons following exposure to RF-EMFs (445 V/m for 1 hr, 1x and 3x)

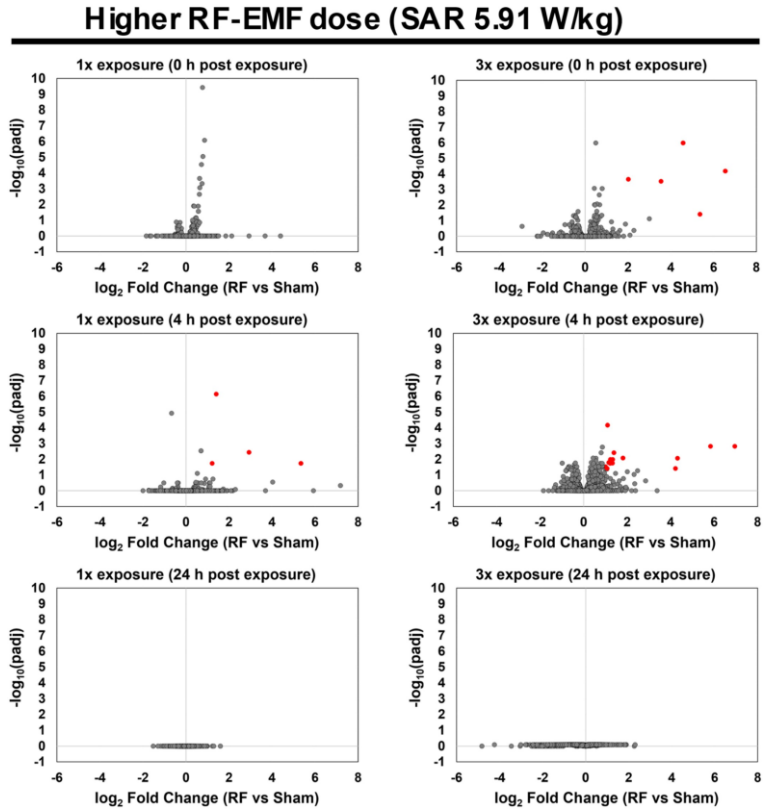


40°C - 41°C



SAR 5.91 W/kg

Differentially expressed genes identified with the higher, thermal (mild heat), RF-EMFs dose



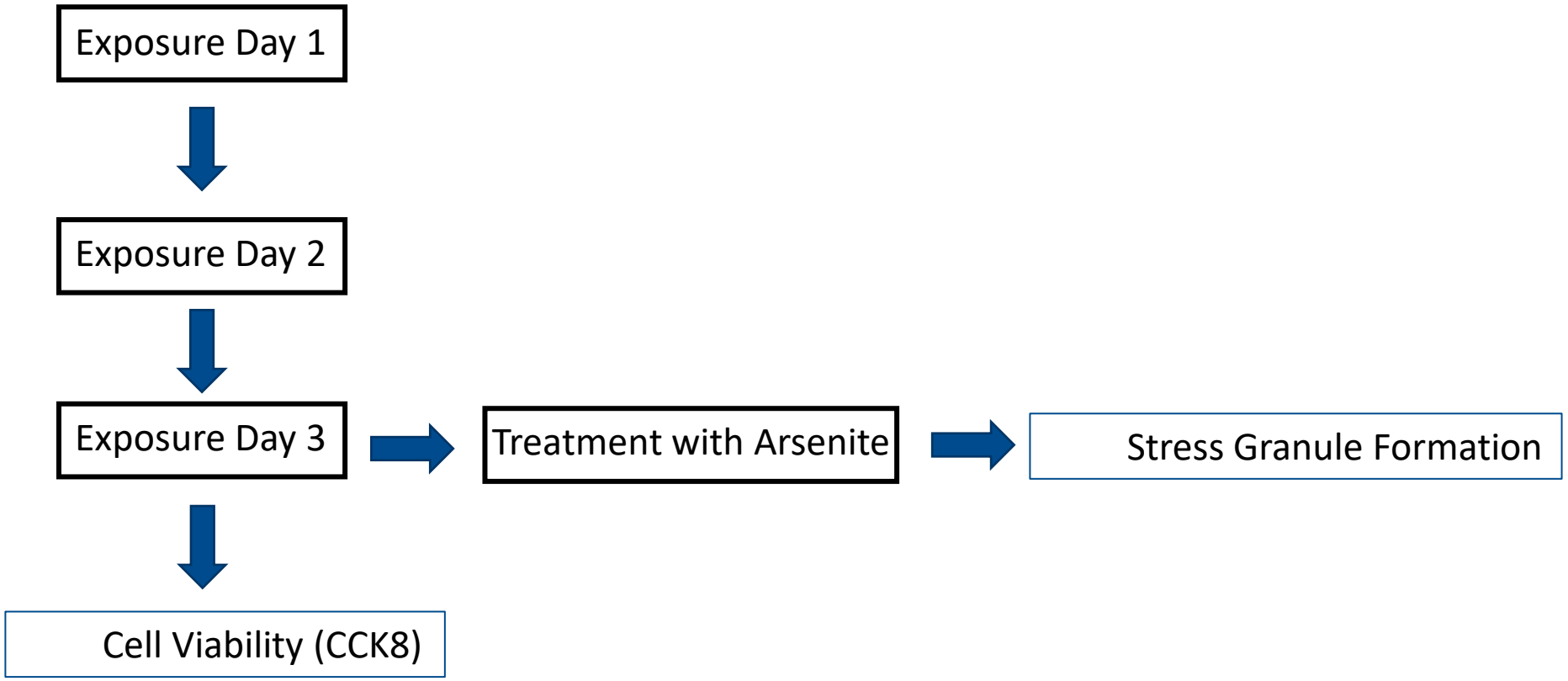
Grey dots are genes that were not significantly different compared to matched shams (at FDR ≥ 0.05, |FC|≤2).

Cantu JC, Butterworth JW, Payne JA, Echchgadda I. Bioelectromagnetics (2024). https://doi.org/10.1002/bem.22517

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

2. Assess if pre-exposure with RF-EMFs (at an AD) would confer resilience to a subsequent exposure to sodium arsenite

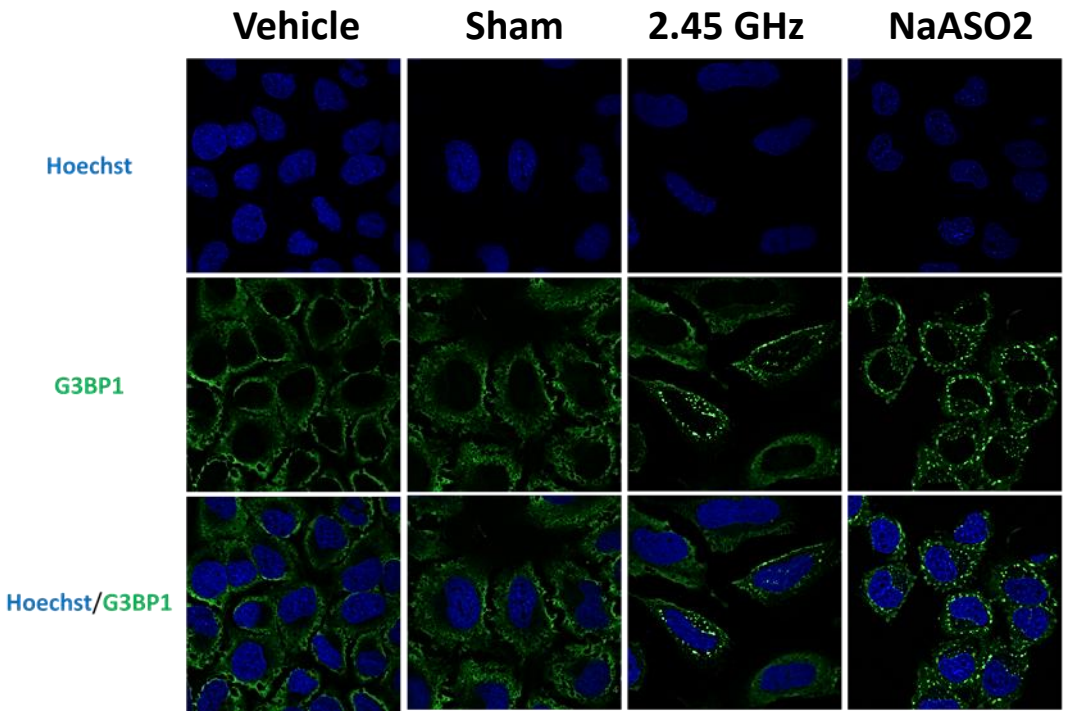
- Effect on cell viability and formation of stress granules in response to sodium arsenite



DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

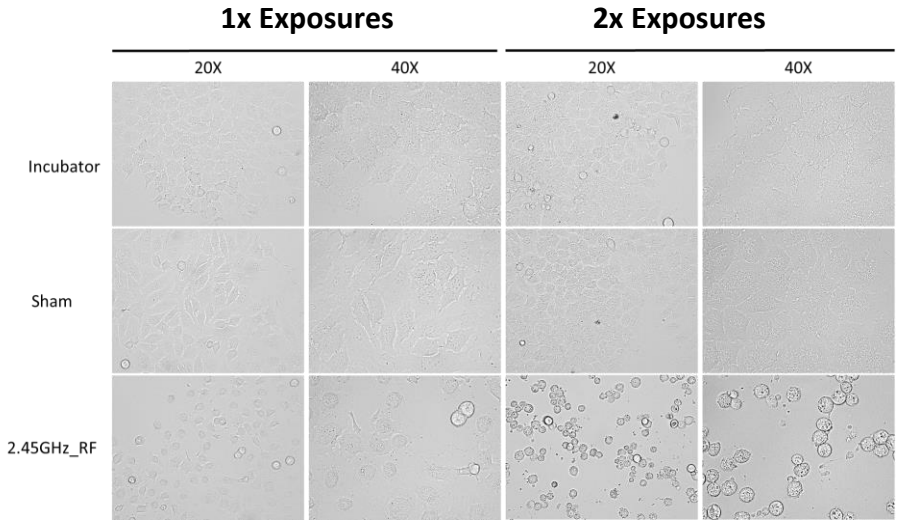
Exposure of HeLa Cells to 2.45 GHz at E-Field of 472 V/m (125W output power)

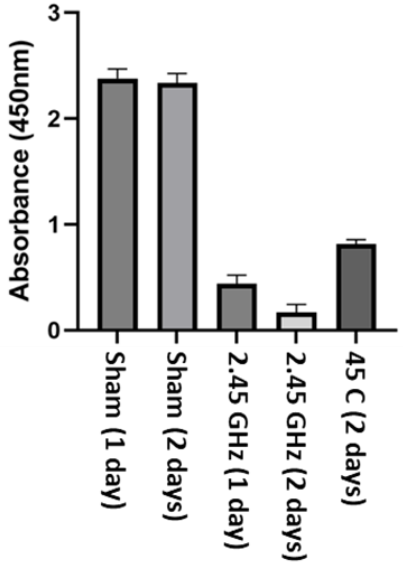
Formation of stress granules

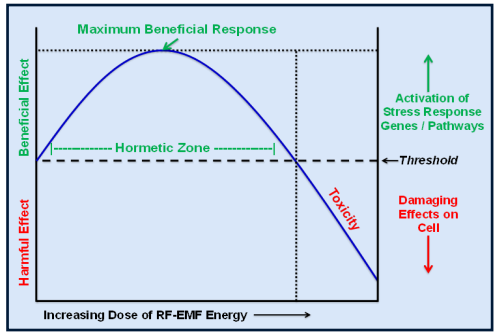


- Exposure Time: 1hr
- Max Temperature: 44°C - 45°C
- Sodium Arsenite ($NaASO_2$) at 0.5mM (1hr)

Cell viability

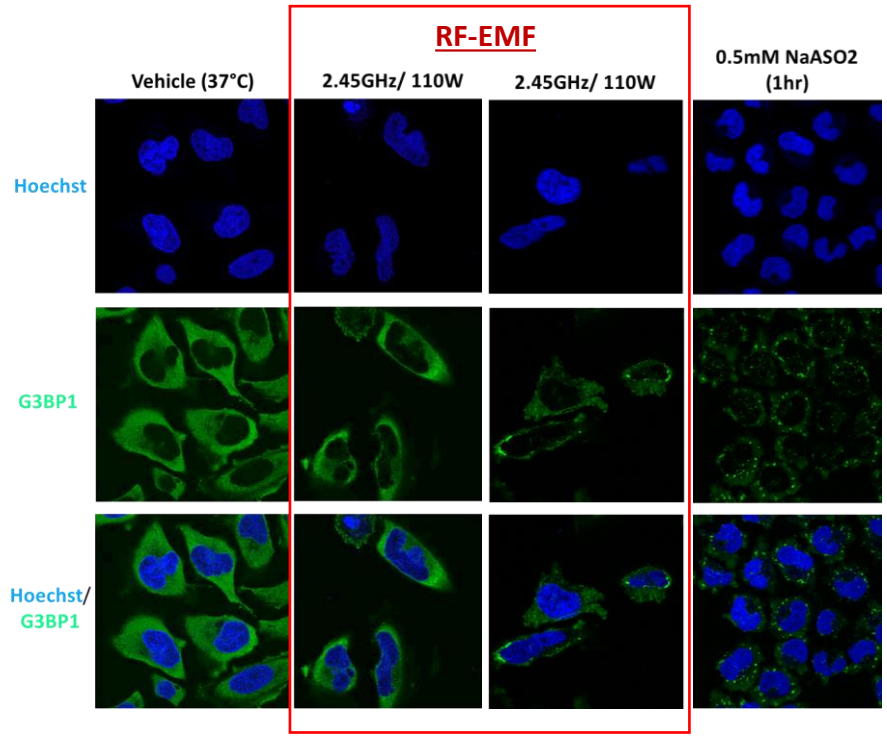


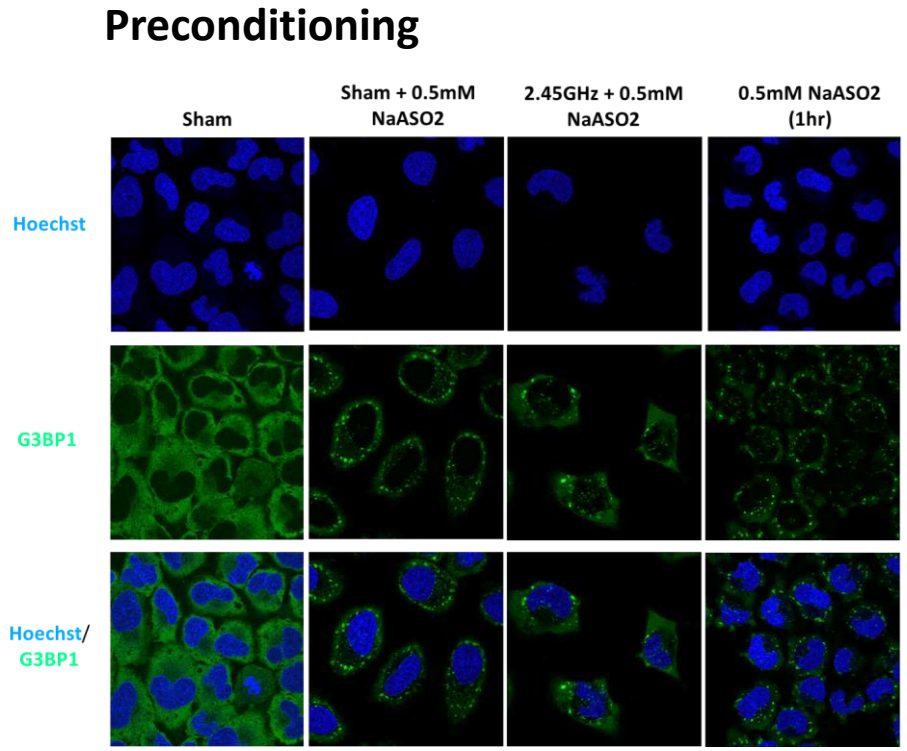




DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Exposure of HeLa Cells to 2.45 GHz at E-Field of 445 V/m (110W output power)



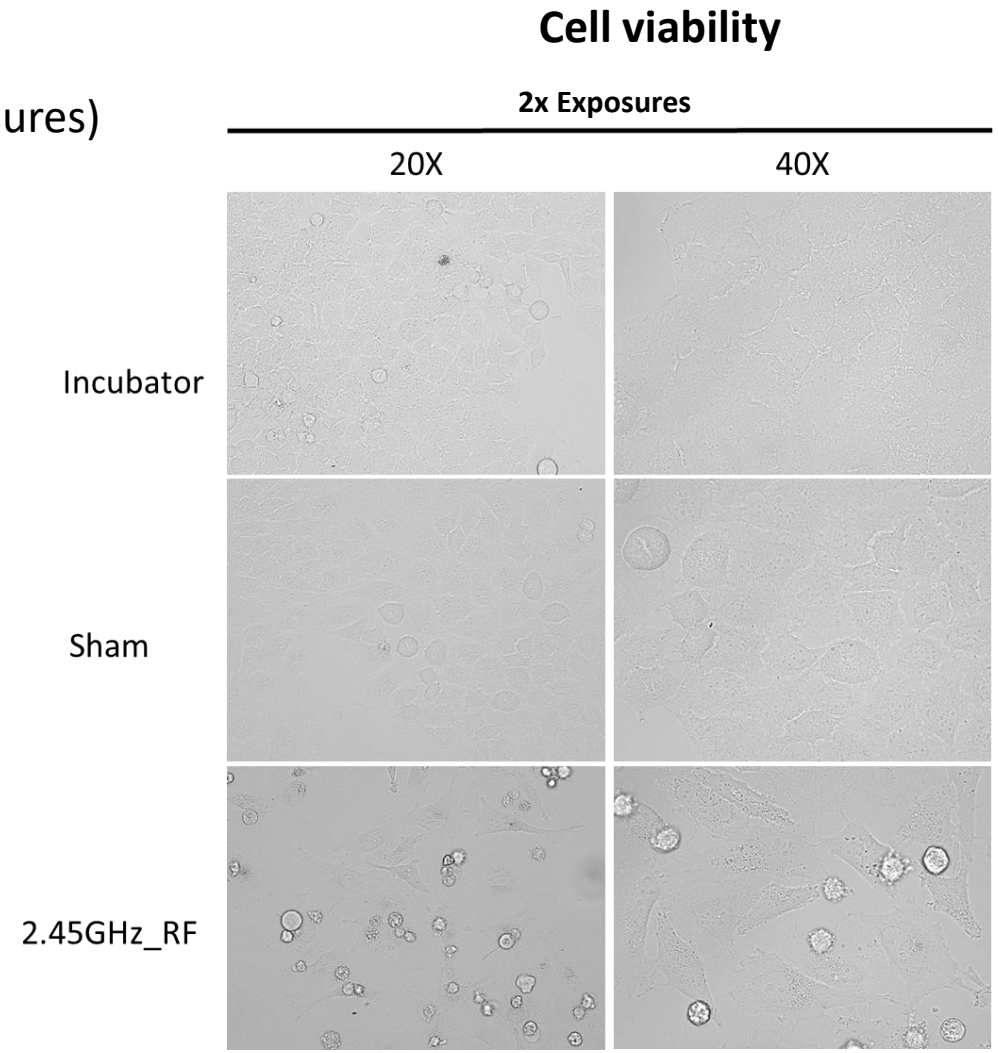


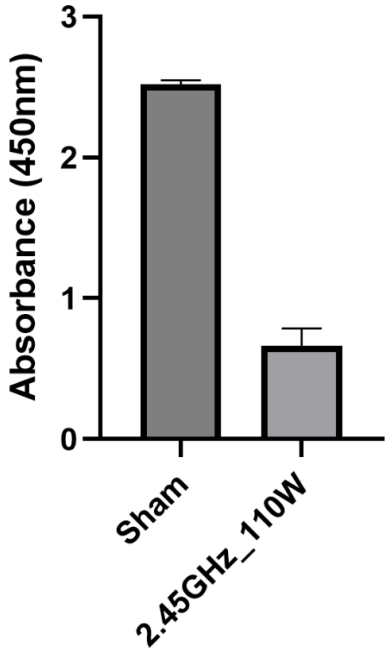
- Exposure Time: 1hr (2x exposures)
- Max Temperature: 40°C -41°C
- Ars ($NaASO_2$) 0.5mM (1hr)

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Exposure of HeLa Cells to 2.45 GHz at E-Field of 445 V/m (110W output power)

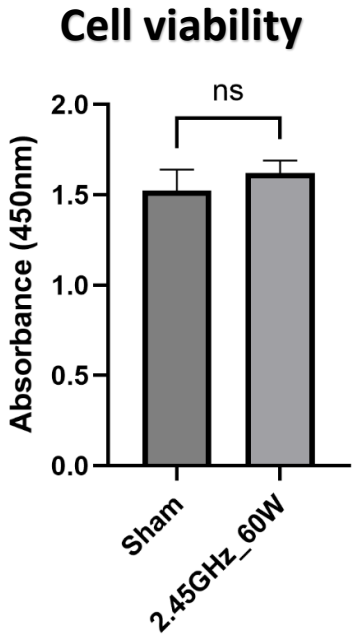
- Exposure Time: 1hr (2x exposures)
- Max Temperature: 40°C -41°C
- Ars ($NaASO_2$) 0.5mM (1hr)

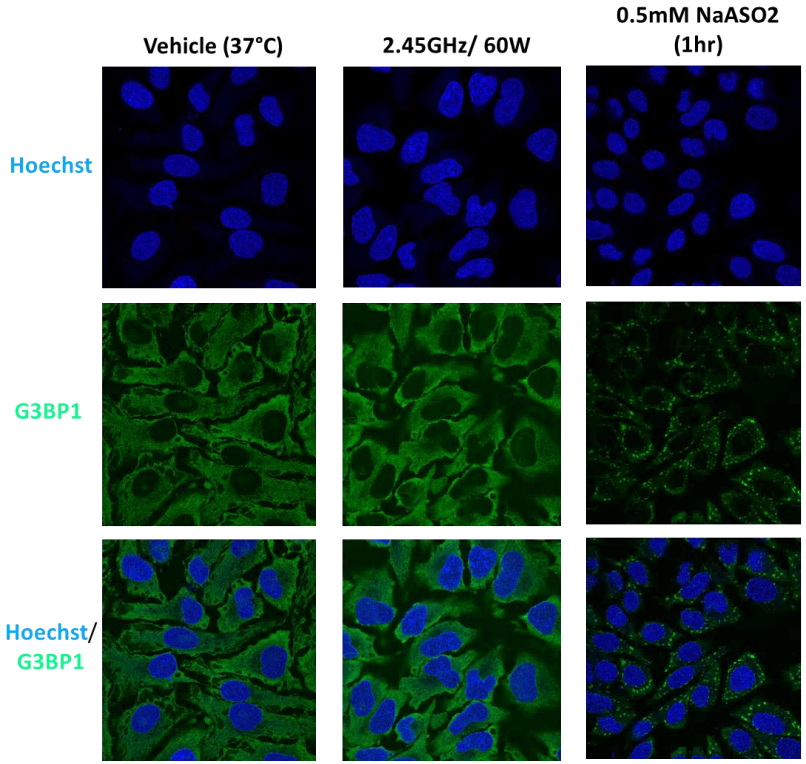


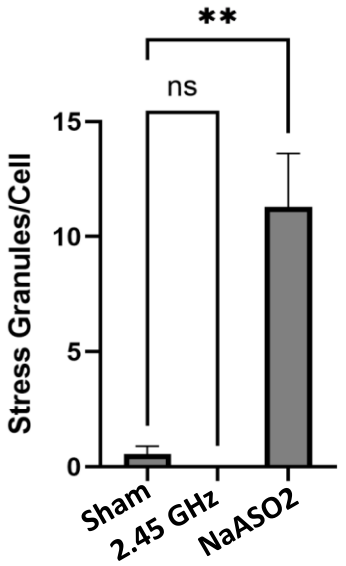


DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Exposure of HeLa Cells to 2.45 GHz at E-Field of 350 V/m (60W output power)







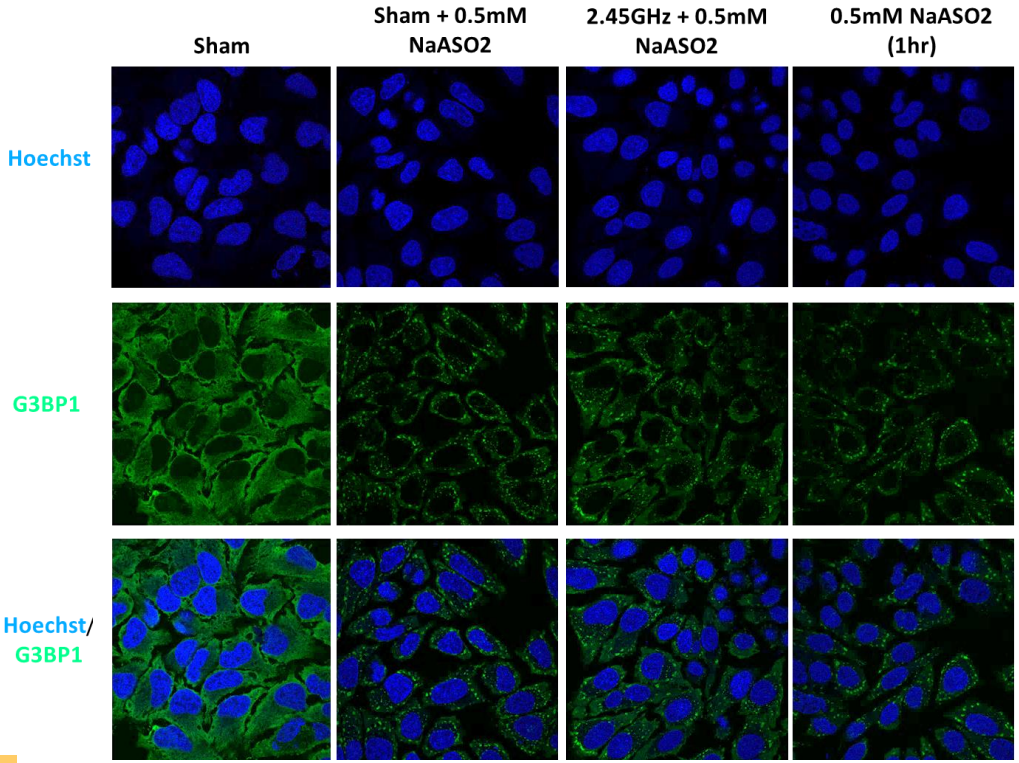
- Exposure Time: 1hr
- Max Temperature: 39°C
- Arsenite treatment immediately post RF-EMF exposure
- Ars (NaASO2) 0.5mM (1hr)

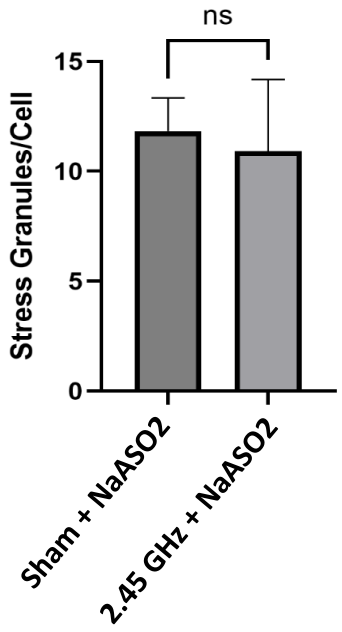
Celleste-6 imaging software

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Exposure of HeLa Cells to 2.45 GHz at E-Field of 350 V/m (60W output power)

Effect of preconditioning on formation of stress granules





Celleste-6 imaging software

- Exposure Time: 1hr
- Max Temperature: 39°C
- Arsenite treatment immediately post RF-EMF exposure
- Ars ($NaASO_2$) 0.5mM (1hr)

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Exposure of HeLa Cells to 2.45 GHz at E-Field of 350 V/m (60W output power)

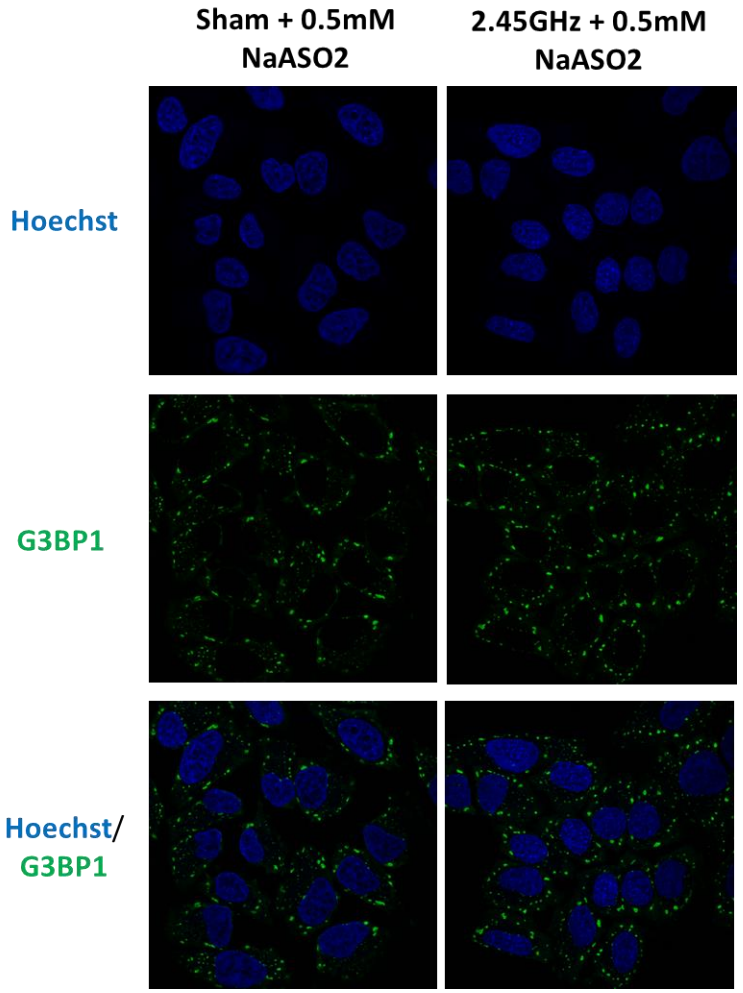
Exposure Parameters

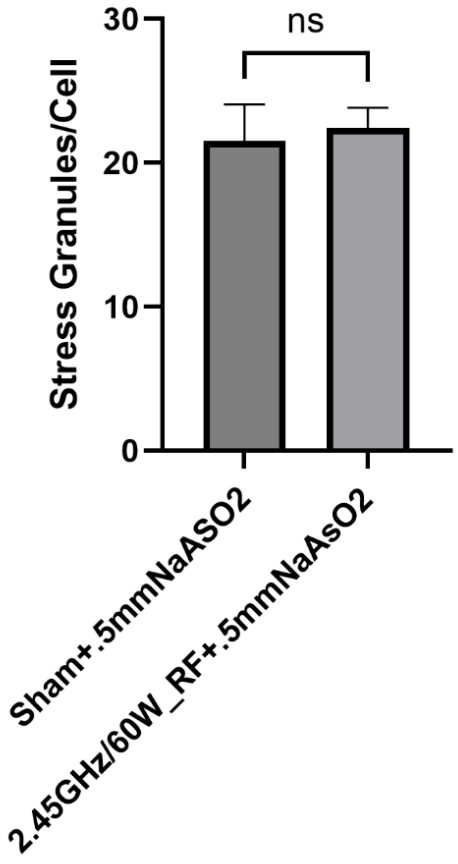
- Frequency: 2.45GHz
- Output Power: 60W
- E-Field: 350 V/m
- Exposure Time: 1hr
- Max Temperature: 39°C

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Exposure of HeLa Cells to 2.45 GHz at E-Field of 350 V/m (60W output power)

Effect of preconditioning on formation of stress granules (4 hours post)

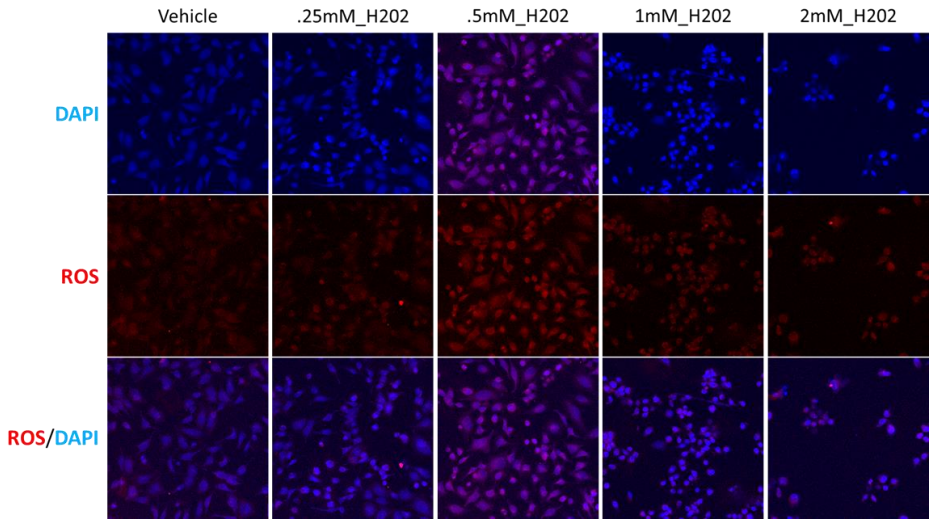


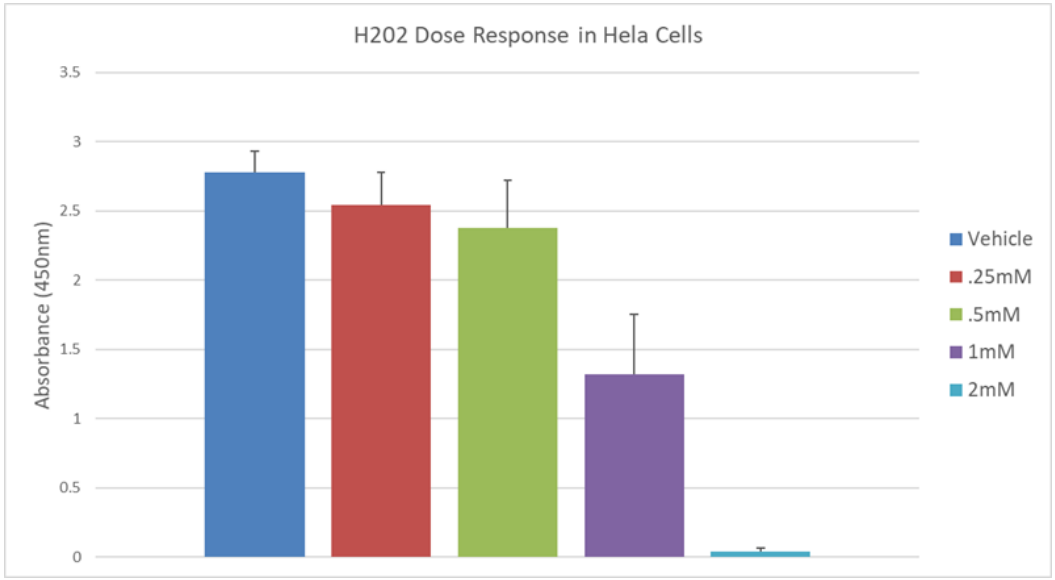


DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Assess if pre-exposure with RF-EMFs (at an AD) would confer resilience to Induced Oxidative Stress -- Ongoing

Reactive Oxygen Species (ROS)





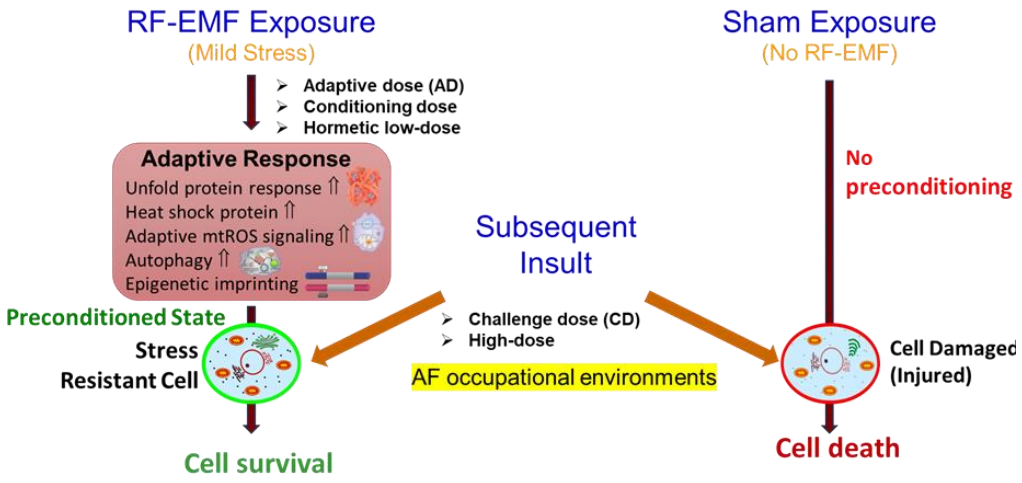
H_2O_2 Dose Response

Does preconditioning (pre-exposure) with an AD of RF-EMFs protects cells from oxidative stress induced by a subsequent exposure to menadione/hydrogen peroxide/sodium arsenite?

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Project Aim 2 – Summary of Results and future directions

- 2. Assess if preconditioning with AD RF-EMFs would confer resilience to a subsequent exposure to stressors



- We have performed experiments examining potential protective effect of CW RF-EMF pre-exposure on cell viability and formation of stress granules
  - So far, our results don't fully support an adaptive response from exposure to CW RF-EMF under the conditions tested, investigating protection from menadione-induced cell death or sodium arsenite-induced stress granules formation
  - More experiments need to be performed to conclude such effect

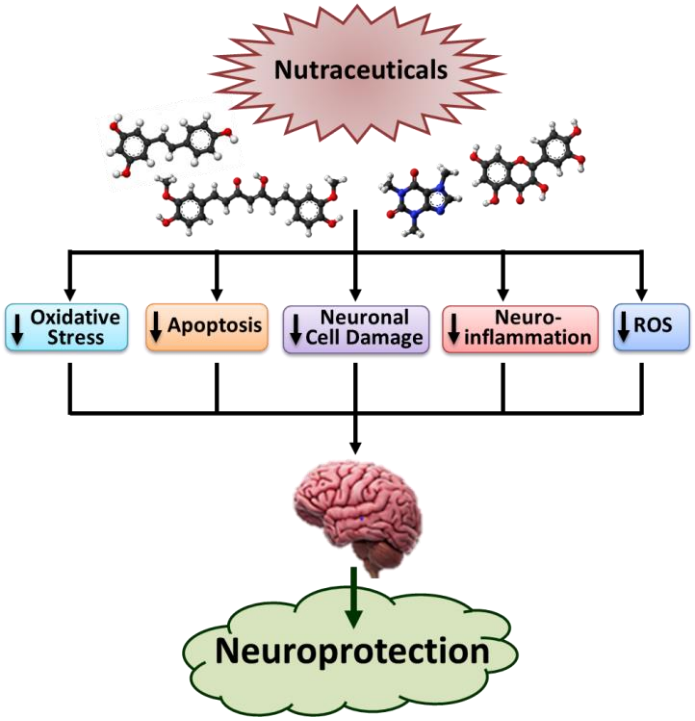
❑ Future experiments:

- Continue testing effects of additional CW exposure conditions, and to include tests from pulsed RF-EMF exposures under similar SAR value doses.
- Assess additional end points, e.g., gene expression, epigenetic imprinting, mitochondria bioenergetics, etc.

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

3. Additive/synergistic effects of RF-EMF combined with neuronal preconditioning agents

Nutraceuticals relevant to neuroprotection



1) Resveratrol
2) Caffeic acid phenethyl ester (CAPE)

Projected to start on FY24 -FY25
Will be evaluated in addition to pre- or post-conditioning with RF-EMF against severe stress

in-vitro ER stress model against oxidative stress

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

FY24-FY25 Future Directions

- Complete the modeling and experimental of RF-EMF exposures for the different exposure conditions
- Continue our investigation of RF-EMF hormetic dose-response in cells
- Continue investigation of preconditioning (pre-exposure) with an AD of RF-EMF and assess protection from subsequent exposure to environmental stressors
- Continue our investigation of the effects of RF-EMFs on the dynamics of microtubules, and cellular state.

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Exploiting Radio Frequency Electromagnetic Field Hormesis to Provide Resilience to Neurons (FY 24) – Dr. Ibtissam Echchgadda

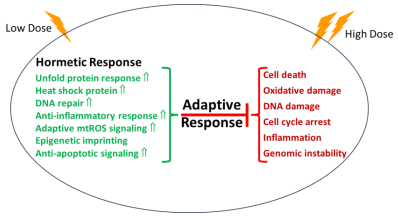
Objectives:

[1] Investigate RF-EMF hormetic dose-response phenomenon

[2] Assess if preconditioning with an adaptive dose (AD) of RF-EMFs would confer adaptive response (AR) and cell resilience to a subsequent exposure to stressors

[3] Investigate additive/synergistic effects of RF-EMF in combination with selected preconditioning agents

Ibtissam Echchgadda, Ph.D.
(711 HPW/RHDR)



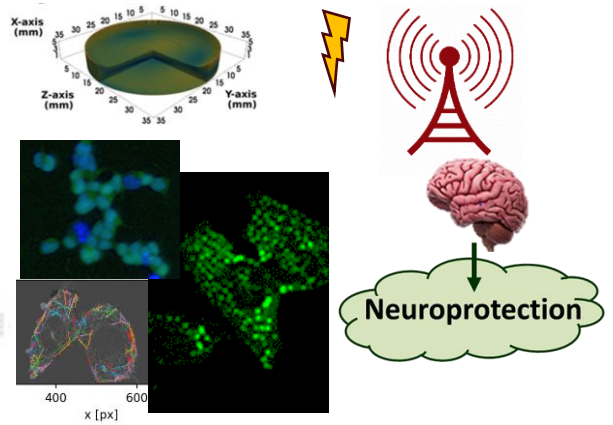
Technical Approach:

Modeling/Dosimetry

RF EMF exposures under reproducible and controlled conditions

Microscopy (imaging) techniques

Cellular and molecular biology methods

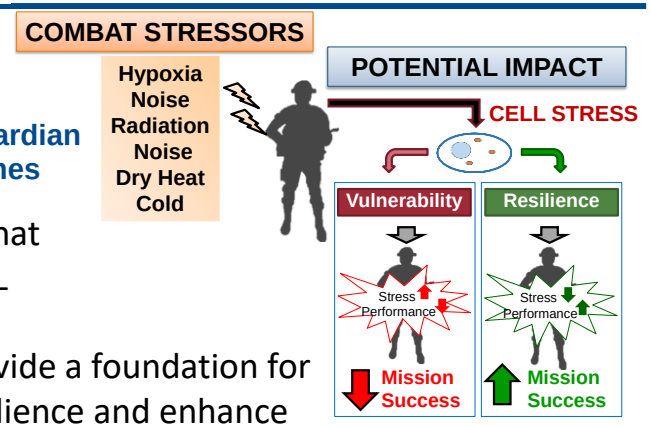


Accomplishments:

- Provided data showing lack of a hormetic dose-response in cells from exposures to continuous wave (CW) RF-EMFs under the conditions tested.
- Examined potential protective effect of CW RF-EMF pre-exposure on cell viability and formation of stress granules. So far, our results don't fully support an AR from exposure to CW RF-EMF under the conditions tested.
- Hired a new Ph.D. student and started a new collaboration with Dr. Romero at UTSA
- Presentations at 2 conferences - BioEM 2024 and FENS 2024
- 1 manuscript published in Bioelectromagnetics

DoD Benefit:

Warfighter and space force guardian stressors and potential outcomes



- Novel aspect of RF-EMF bioeffects that needs to be addressed by DOD/AFRL
- Validation of RF Hormesis could provide a foundation for development of tools to convey resilience and enhance performance of warfighters and space force guardians.
- RF-EMF as an agent for resilience present superiority compared to other preconditioning method (no notable genotoxic effects)

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Acknowledgements

711 HPW/RHDR

- ❑ Dr. Joseph Butterworth (AFRL, 711 HPW/RHDR)
- ❑ Dr. Richard De La Rosa (ORISE postdoc/GDIT)
- ❑ Dr. Jorge Gomez (GDIT)
- ❑ Ms. Kristen Vokt (GDIT)
- ❑ Dr. Gary Newman (ORISE Ph.D. student at UTSA)
- ❑ Dr. Nicholas Mennona (ORISE Ph.D. student at UMD)

COLLABORATORS:

- ❑ Dr. Wolfgang Losert (Institute for Physical Science & Technology, UMD)
- ❑ Dr. Esteban Wright (Institute for Physical Science & Technology, UMD)
- ❑ Dr. Gabriela Romero Uribe (Biomedical Engineering & Chemical Engineering, UTSA)

- ❖ Dr. Patrick Bradshaw

AFOSR
AIR FORCE OFFICE OF SCIENTIFIC RESEARCH

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.

Thank you

DISTRIBUTION STATEMENT A: Approved for public release, distribution unlimited, PA # AFRL 2024-5317, 26 Sept 2024.