

Low-Dose Curcumin Nanoparticle Therapy Improves Brain Function in a Rat Model of Chronic Gulf War Illness

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Introduction

- Cognitive and mood impairments are the most conspicuous CNS symptoms of GWI.
- Similar symptoms are seen in a rat model of GWI (*Parihar et al., 2013; Hattiangady et al., 2014*).
- The model is based on perceived exposures to the GW-related chemicals and stress during the Gulf war

Classic neuropathology in a rat model of GWI

- Persistent cognitive and mood dysfunction (*Parihar et al., 2013, Madhu et al., 2019; Shetty et al., 2020*)
- Incessantly elevated oxidative stress and mitochondrial dysfunction in the brain (*Shetty et al., 2017, 2020*)
- Activated microglia and reactive astrocytes - neuroinflammation (*Madhu et al., 2019; Shetty et al., 2020*)
- Waned neurogenesis in the hippocampus (*Parihar et al., 2013, Kodali et al., 2018*)
- Increased levels of proinflammatory cytokines in the circulating blood (*Shetty et al., 2017, 2020*)

Therefore, testing the efficacy of systemic administration of compounds having robust antioxidant and/or antiinflammatory activity has received significant attention.

Hypothesis

Low dose curcumin nanoparticle (nCUR) therapy is sufficient for alleviating cognitive and mood impairments in a model of chronic GWI with robust antioxidant and antiinflammatory effects

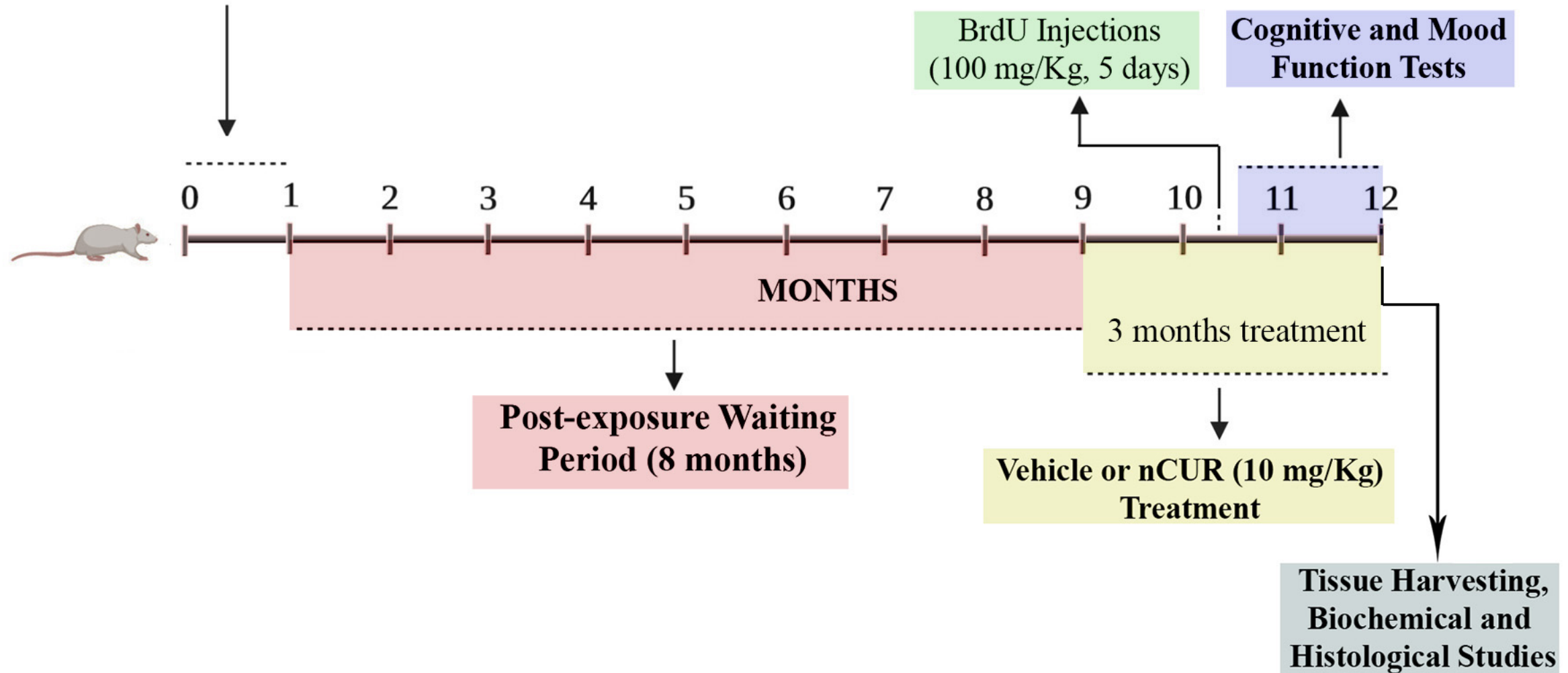
Questions addressed

- Would low-dose (10 mg/Kg) and intermittent oral administration (3 days/week) of curcumin nanoparticles (nCUR) to rats with chronic GWI (commencing 8 months after exposure to GW-related chemicals and stress):
 - Improve cognitive and mood function?
 - Reduce oxidative stress and improve mitochondrial function in the hippocampus?
 - Diminish inflammation and NLRP3 inflammasomes in the hippocampus?
 - Enhance hippocampal neurogenesis?

Research Design

**Exposure to
GWI-related Chemicals
and Stress (28 days)**

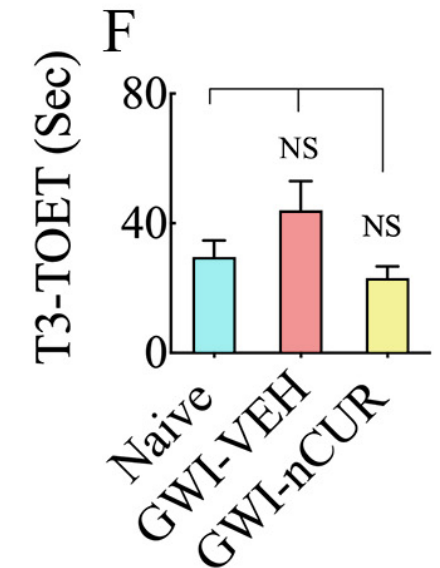
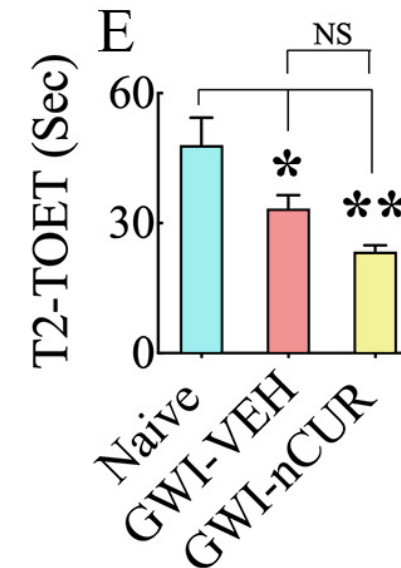
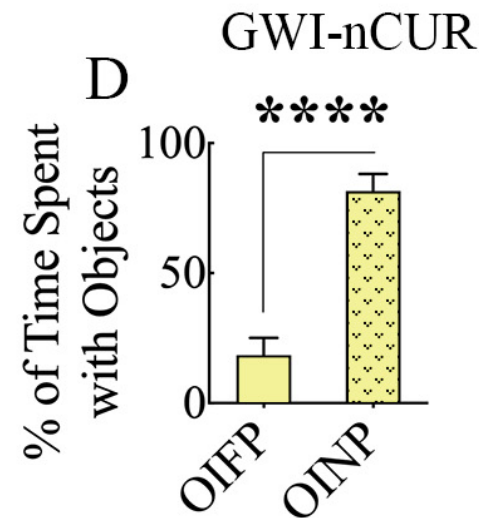
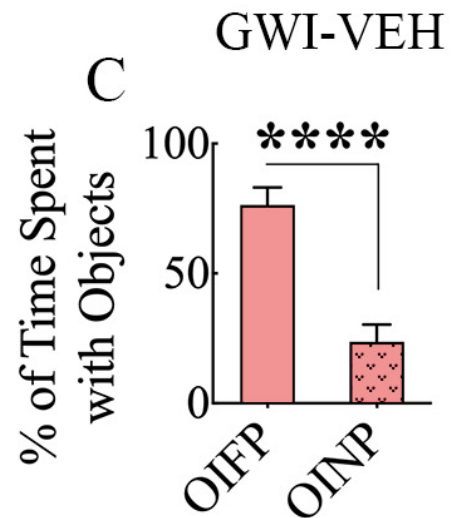
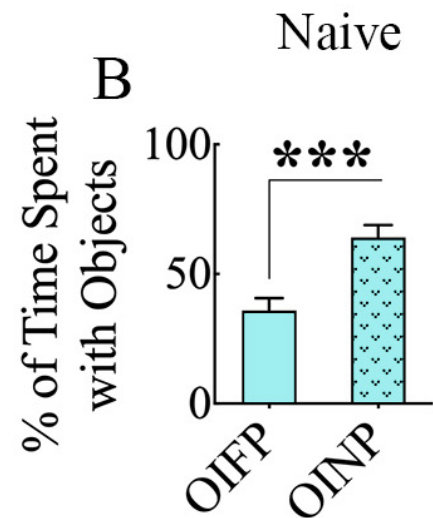
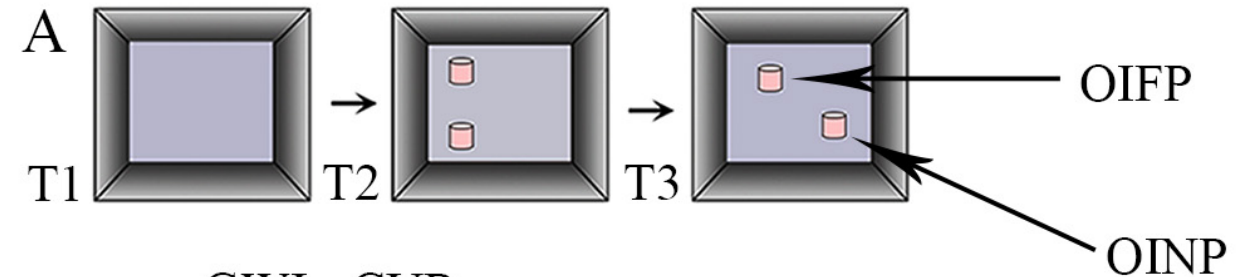
**PB, DEET, PER and
15 min restraint stress**



Hippocampus-Dependent Object Location Test (OLT)

- Rats with chronic GWI displayed inability to discern a minor change in their environment.
- nCUR treatment to GWI rats improved this hippocampus-dependent cognitive function.

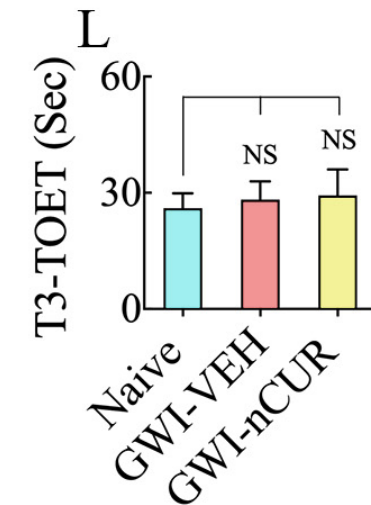
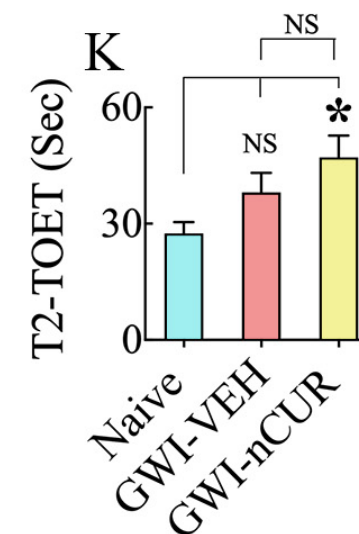
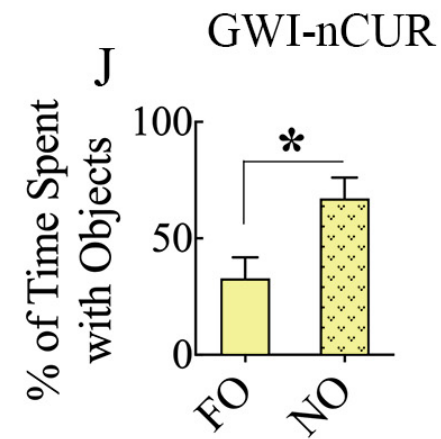
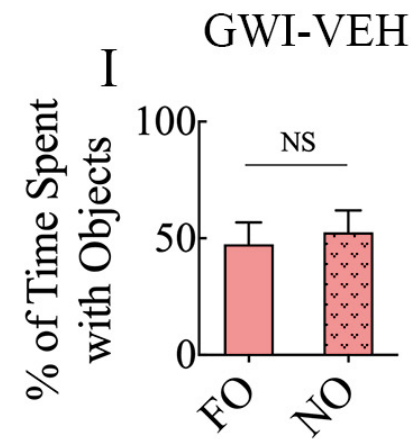
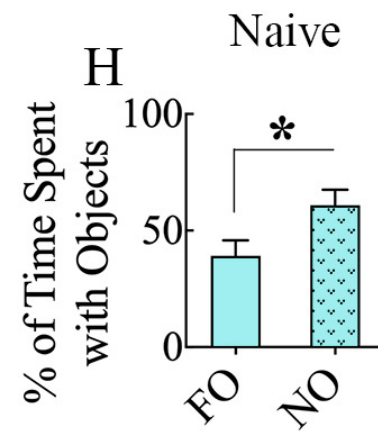
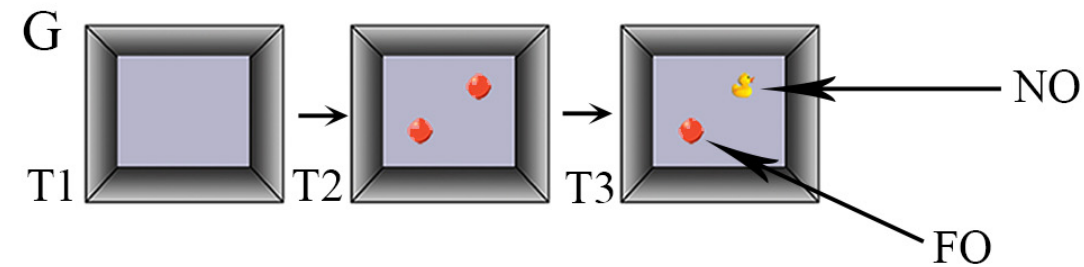
Object Location Test (OLT)



Novel Object Recognition Test (NORT)

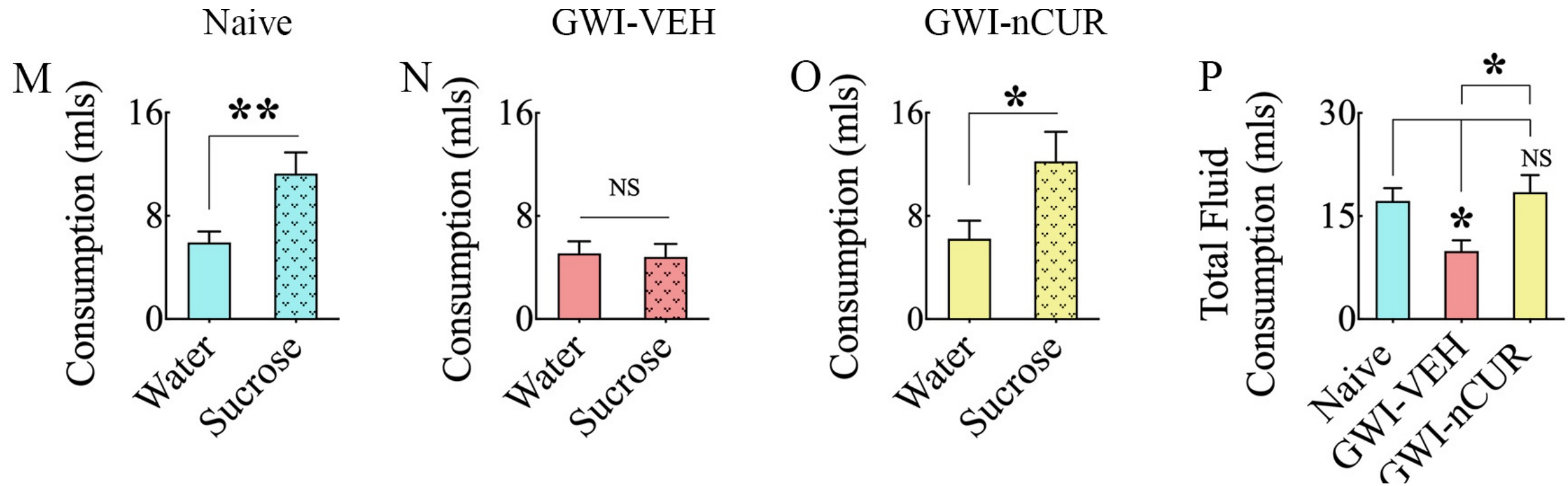
- Rats with chronic GWI displayed recognition memory impairment.
- nCUR treatment to GWI rats improved this function dependent on the integrity of perirhinal cortex and hippocampus.

Novel Object Recognition Test (NORT)



Sucrose preference test (SPT)

- GWI rats exhibited anhedonia (i.e., inability to feel pleasure in activities that are pleasurable in healthy conditions, a measure of depression).
- nCUR treatment to GWI rats reversed anhedonia.

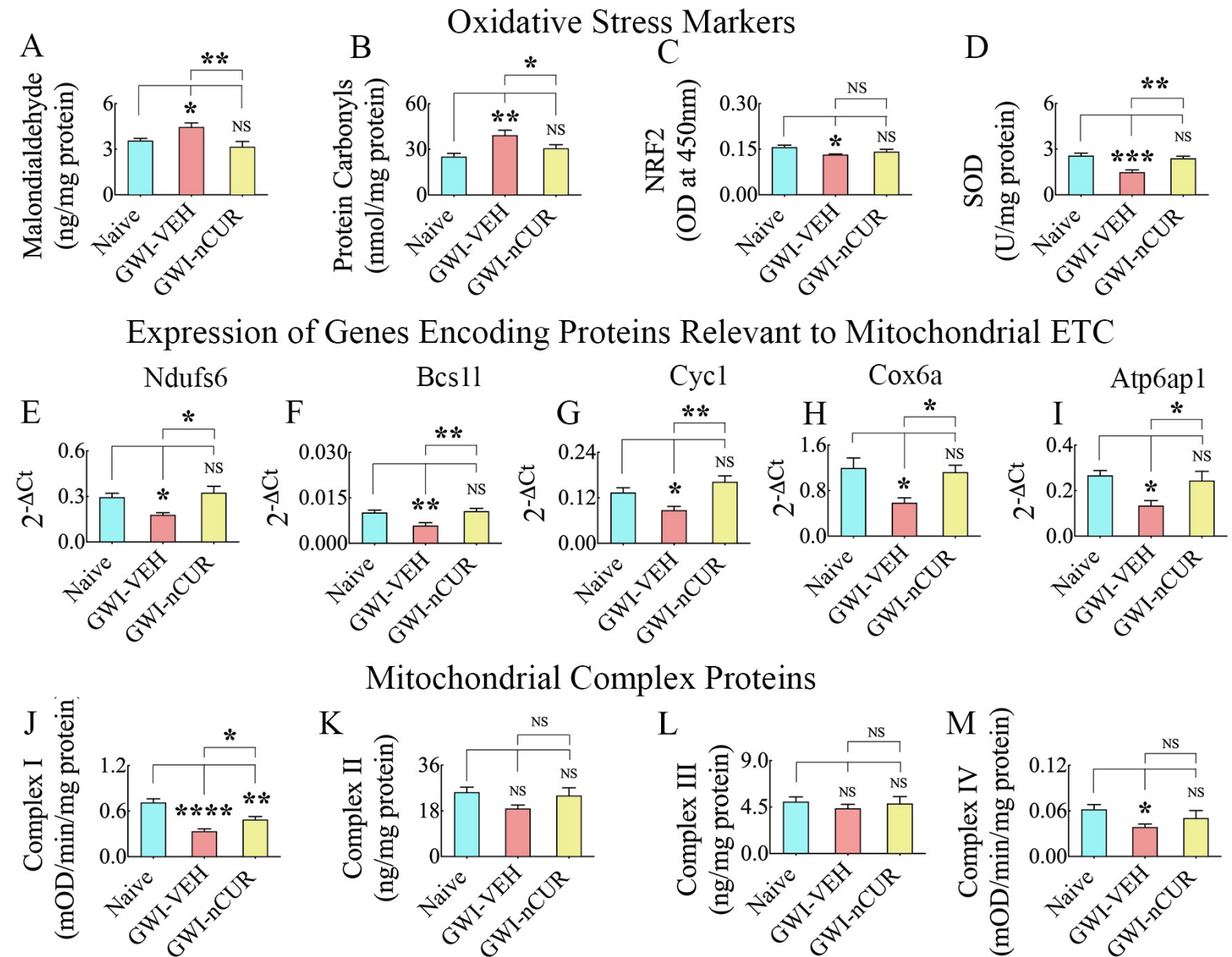


Twelve weeks of nCUR Treatment to GWI Rats Reduced Oxidative Stress, and Improved Mitochondrial Function

- nCUR treatment normalized:

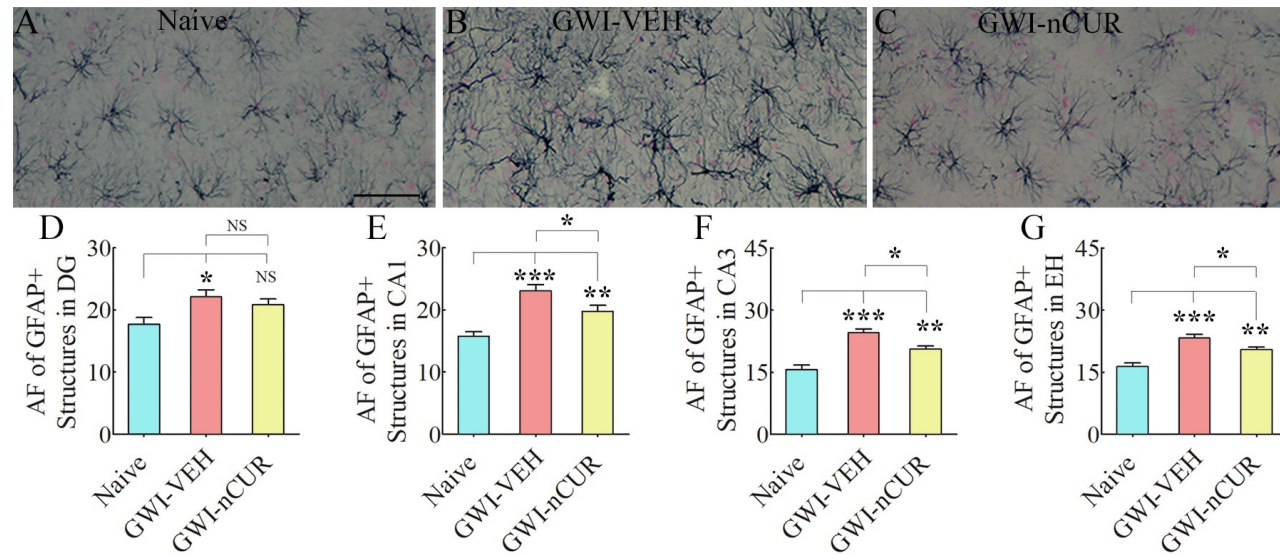
- Malondialdehyde (MDA)
- Protein carbonyls (PC)
- NRF-2
- Superoxide dismutase (SOD)
- Mitochondrial gene expression

nCUR treatment also increased the concentration of Complex I and Complex IV

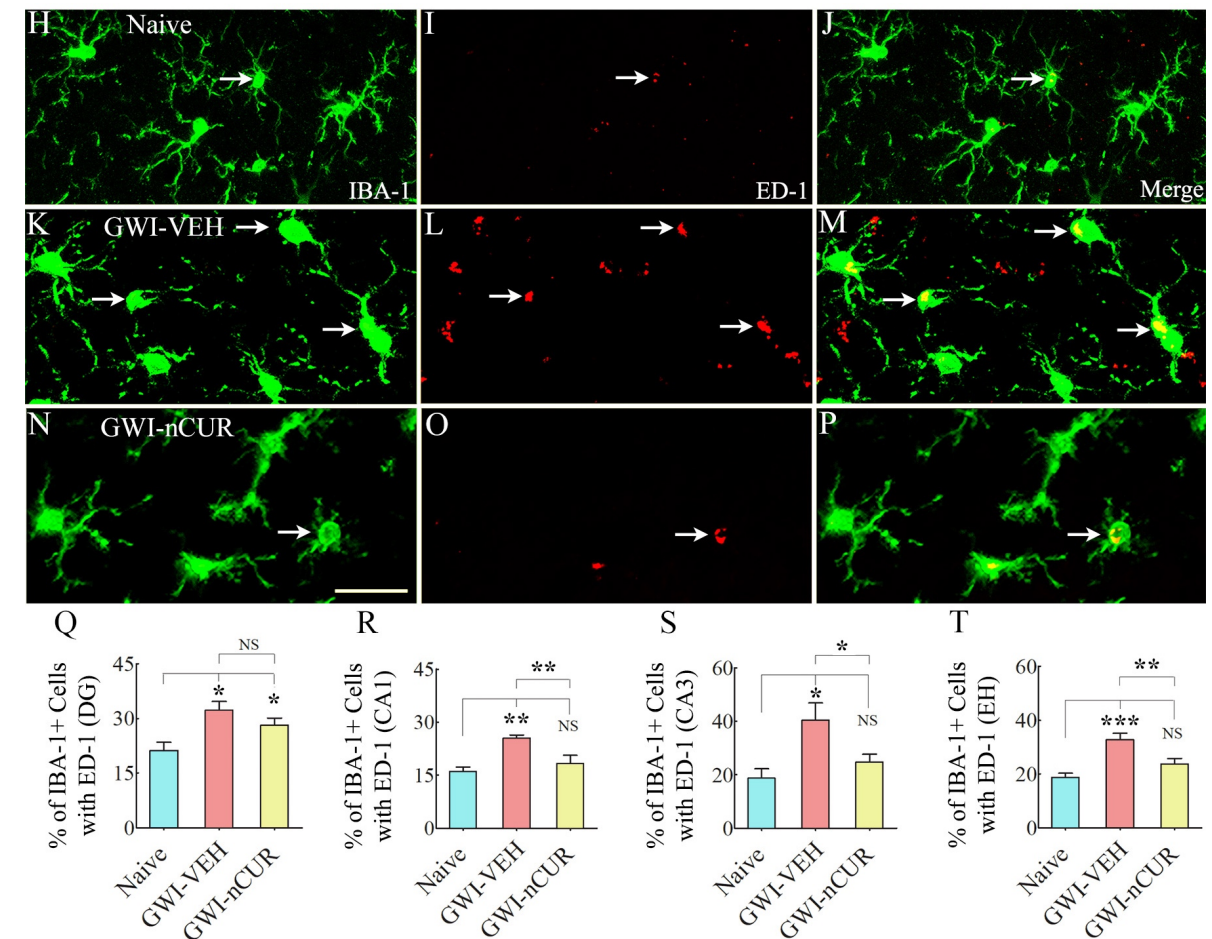


Twelve weeks of nCUR Treatment to GWI Rats Reduced Astrocyte Hypertrophy and Activated Microglia in the Hippocampus

Reactive Astrocytes (GFAP)

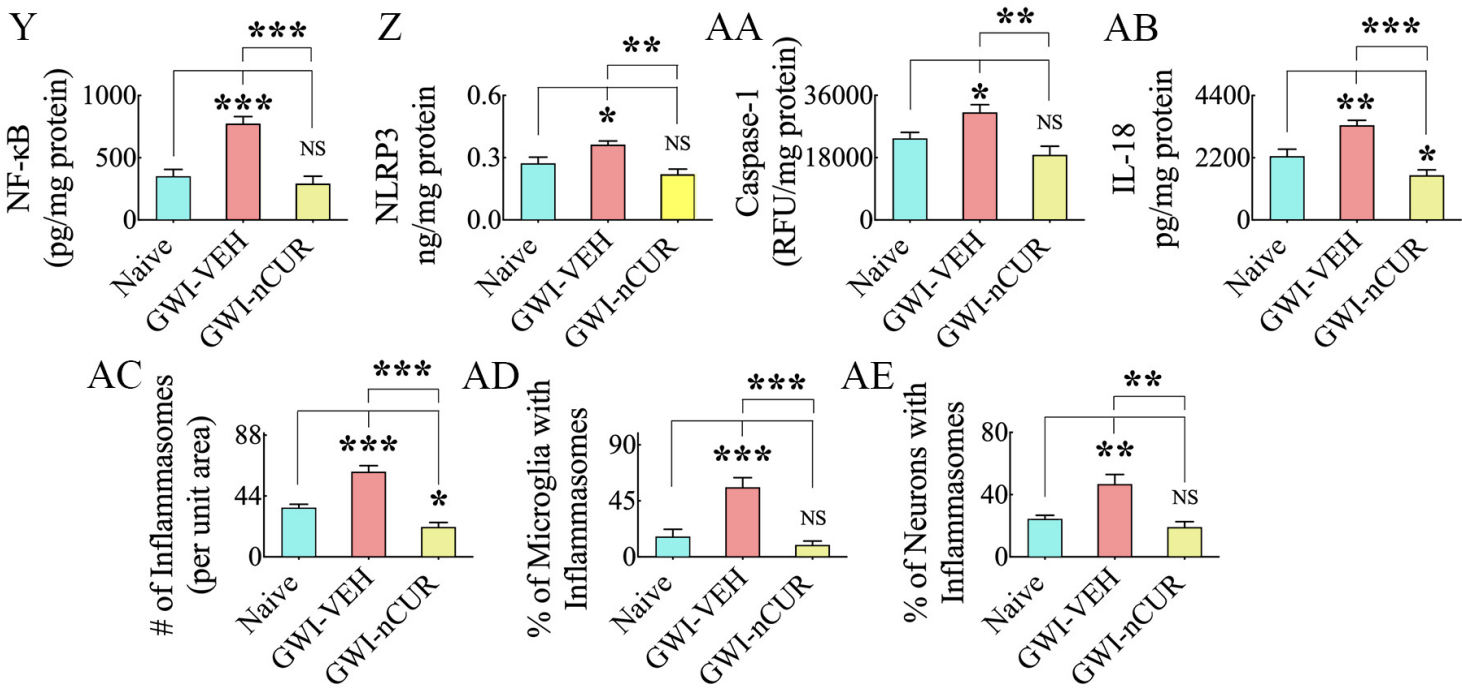
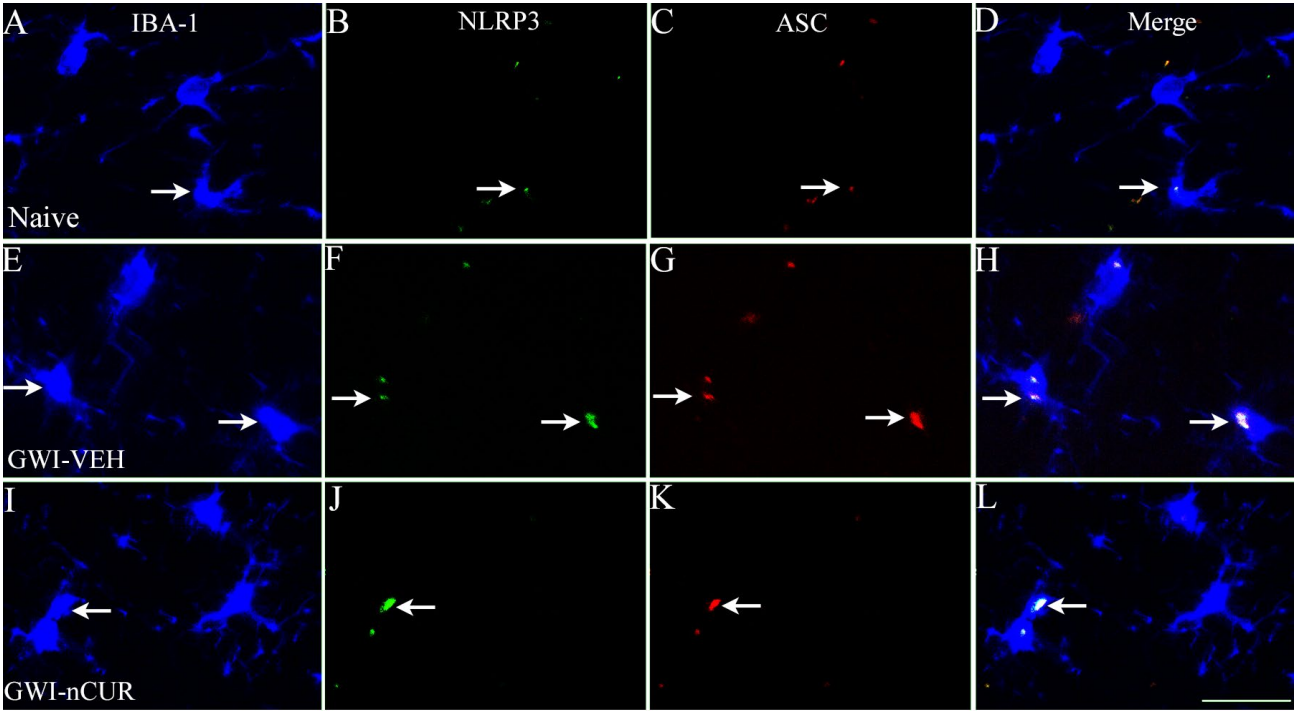


Activated Microglia (IBA-1 & ED-1)



Twelve weeks of nCUR Treatment to GWI Rats Reduced Microglia with NLRP3 Inflammasomes in the Hippocampus

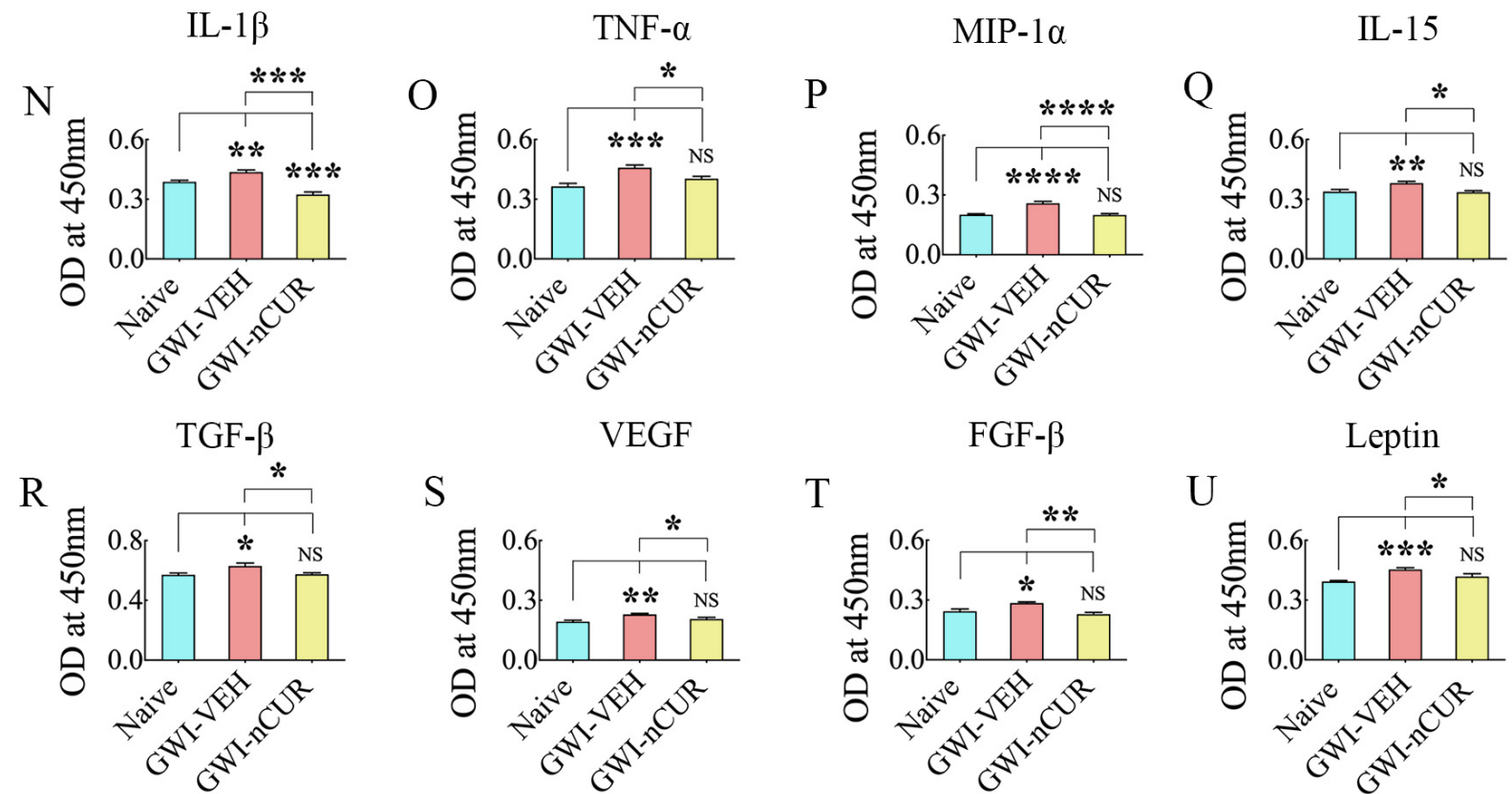
IBA-1, NLRP3, ASC



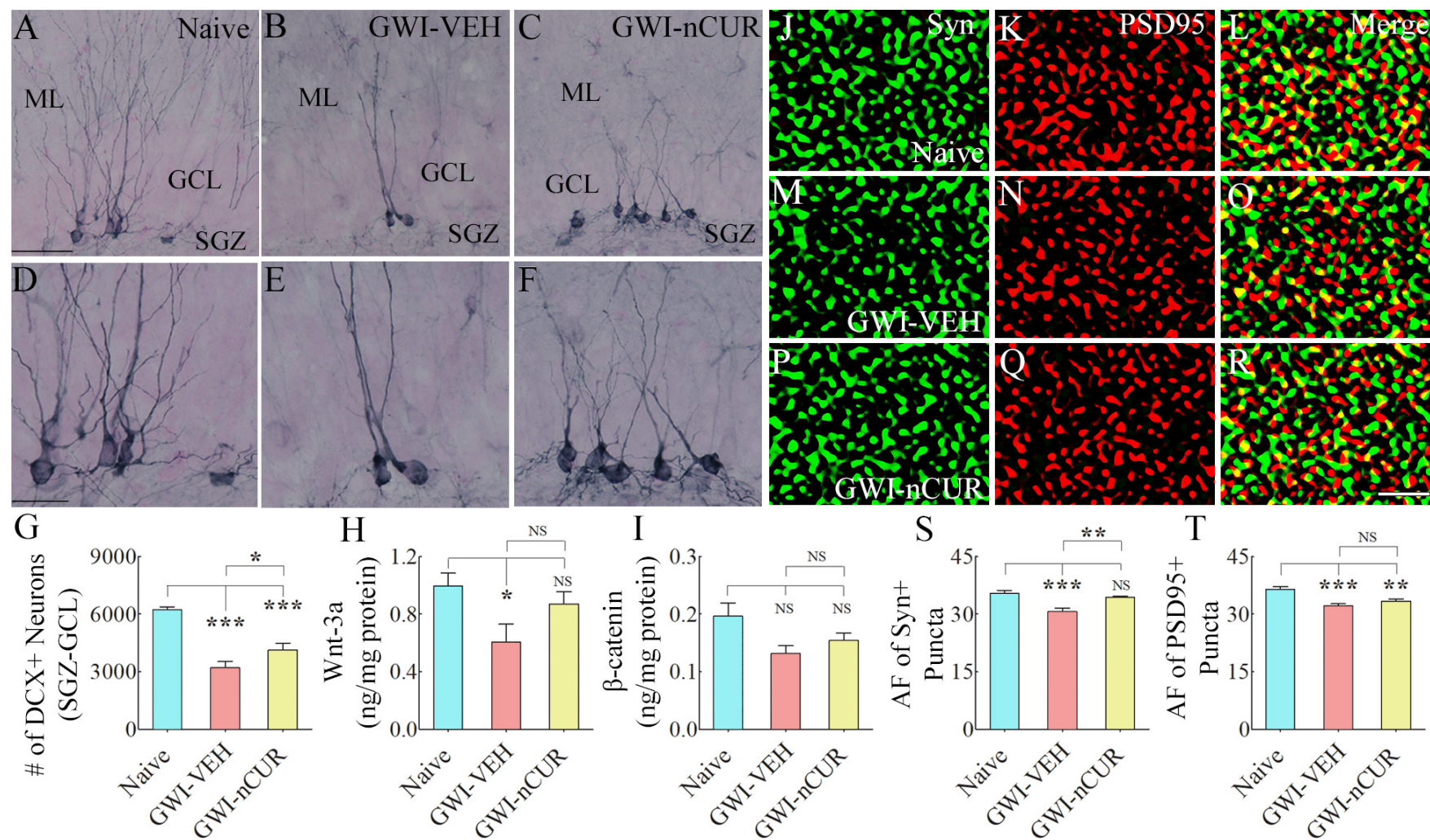
Twelve weeks of nCUR Treatment to GWI Rats Diminished the Concentration of Multiple Proinflammatory Markers in the Hippocampus

- GWI rats displayed increased concentration of multiple proinflammatory cytokines in the hippocampus, at ~11 months post-exposure.
- The inflammatory mediators included IL-1 β , TNF α , MiP-1 α , IL15, TGF- β , VEGF, and Leptin.
- Twelve weeks of nCUR treatment normalized the concentration of the inflammatory mediators in the hippocampus.

Levels of Pro-inflammatory Cytokines and Chemokines

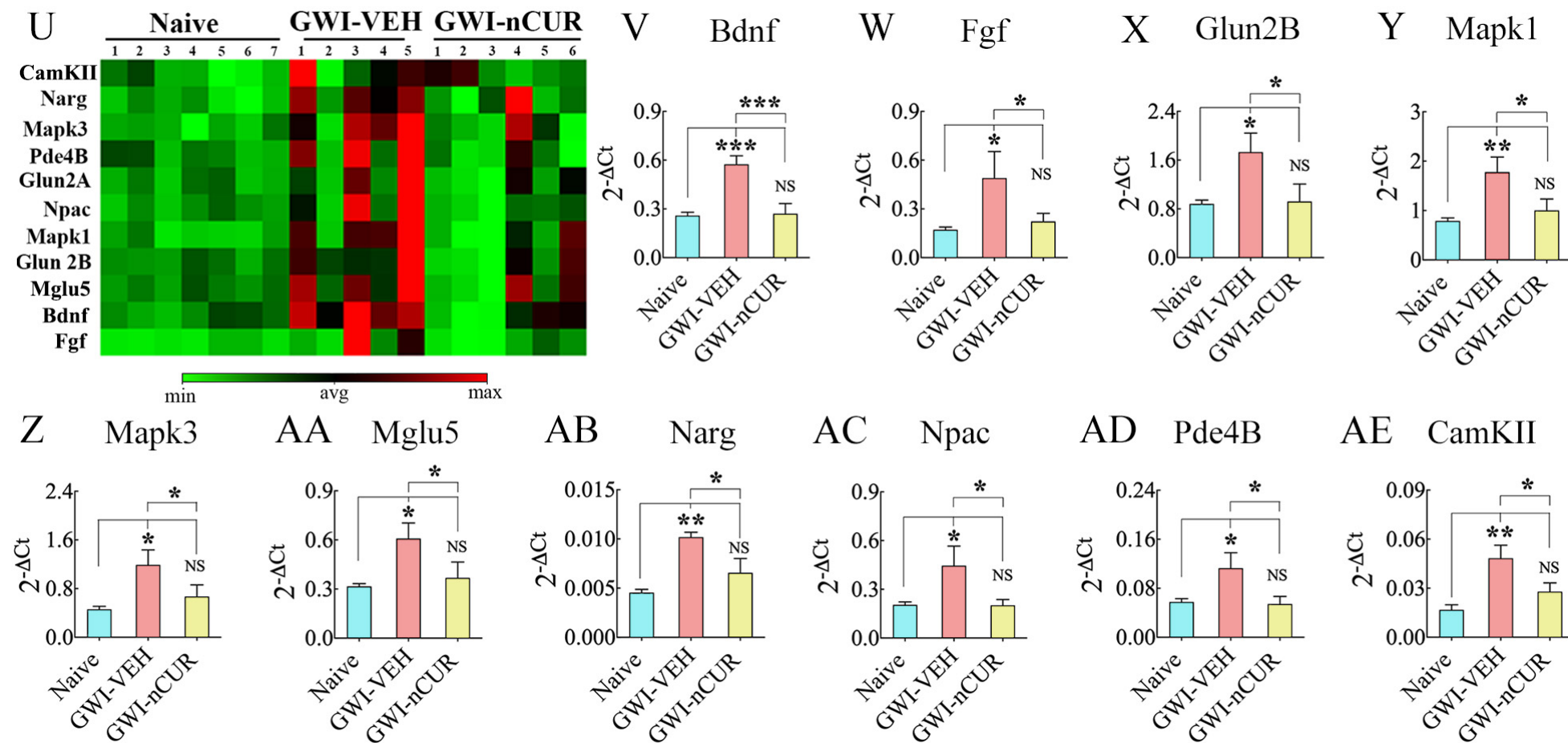


Twelve Weeks of nCUR Treatment to GWI Rats Increased Hippocampal Neurogenesis, and Increased Synaptophysin Density



Twelve weeks of nCUR Treatment to GWI Rats Normalized the Expression of Genes Involved in Cognitive Function in the Hippocampus

Expression of Genes Encoding Proteins Relevant to Cognitive Function



Conclusions

Twelve weeks of low dose, intermittent, oral nCUR treatment to rats displaying chronic GWI:

- **Improved cognitive function** – *evident from the improved ability for perceiving minor changes in the environment, and novel object recognition.*
- **Improved Mood function** – *evident from the reversal of anhedonia.*
- **Diminished Oxidative Stress in the Hippocampus** – *apparent from the decreased concentration of oxidative stress markers, and increased concentration of NRF-2 and SOD in the hippocampus.*
- **Improved mitochondrial activity** – *gleaned from the normalized expression of genes encoding proteins linked to mitochondrial ETC, and mitochondrial complex proteins in the hippocampus.*
- **Diminished neuroinflammation** – *evident from the decreased concentration of various inflammatory mediators, diminished hypertrophy of astrocytes, reduced density of activated microglia with NLRP3 inflammasomes.*
- **Enhanced hippocampal Neurogenesis** – *evident from increased numbers of DCX+ newly born neurons.*
- **Normalized the expression of multiple genes that encode proteins involved in cognitive function.**

Thus, low dose, intermittent oral nCUR treatment could improve brain function in veterans with GWI.

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Shetty LAB

(College of Medicine)

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nCUR production and Characterization

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