

Neural Stem Cell Dysfunction & Its Implications on Memory and Mood in a Rat Model of Gulf War Illness

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Gulf War illness (GWI)

Affected population

Veterans who served in the 1991 Persian Gulf War-1 (PGW-1)
[~25% of 697,000 US Servicemen & women]

Symptoms

A set of non-specific concurrent symptoms
with an emphasis on CNS impairments

- Memory and Concentration Problems
- Depression, Anxiety, and Chronic headaches
- Dizziness & Alterations in Sleep, widespread pain etc.

Possible Causes of Gulf War Illness

Exposure to a Mixture of Biological and Chemical Environments during PGW-1

(1) Intake of Pyridostigmine bromide (PB)

As a Prophylactic measure against nerve gas attack

(2) Exposure to N, N-diethyl-m-toluamide (DEET) & Permethrin

To Combat insects and rodents in the region

Other suspected factors:

Low-level exposure to nerve gas agents, proximity to oil-well fires, receipt of multiple vaccines, and effects of combinations of Gulf war exposures etc..

Based on the report of “The Research Advisory Committee on Gulf War Veterans' Illnesses”

The symptoms exhibited by Gulf war veterans are likely owed to a synergistic interaction of chemicals PB, and pesticides (such as DEET and permethrin).

Rat model of Gulf War Syndrome

Exposure of rats for prolonged periods (e.g. 28 days) to low doses of PB, DEET & Permethrin

PB: 1.3 mg/kg/day, oral

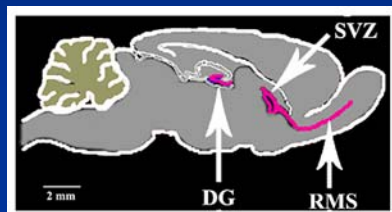
DEET: 40 mg/kg/day, dermal

Permethrin: 0.13 mg/kg/day, dermal

Experiment #1

Immediate Effects of 28-Day Exposure to Chemicals PB, DEET, and Permethrin on Hippocampal Neurogenesis

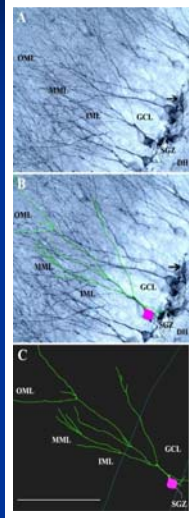
Neurogenic Regions in the Adult Brain



**Dentate Gyrus
&
Subventricular Zone**

Neurogenesis in Non-Neurogenic Regions
Cerebral Cortex, Striatum, Substantia Nigra etc.

Dentate Neurogenesis in the Adult Hippocampus



Rao & Shetty,
Eur. J. Neurosci. 2004

- Production of new neurons in the DG occurs throughout life.
- Newly generated neurons mature into functional neurons.
- Extent of dentate neurogenesis in the adult depends on multiple positive and negative regulators.

Cell death

Concentration of stem/progenitor cell proliferation factors (FGF-2, IGF-1, VEGF, EGF, BDNF)

Serotonin

Enriched environment, exercise, learning & memory training

Vascular niche

Glucocorticoids (Stress)

Hippocampal inflammation

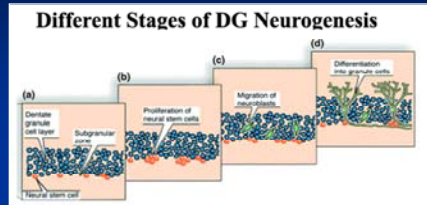
Aging

Drugs of abuse (e.g. alcohol)

Functions of Dentate Neurogenesis

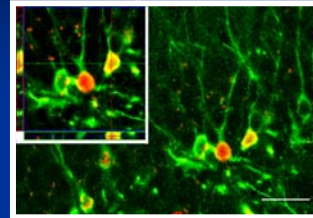
- DG neurogenesis and hippocampal-dependent learning and memory.
- New neurons incorporate into dentate gyrus circuits supporting spatial memory (*Kee et al., 2007*).
- Genetic Ablation of Newly Formed Neurons leads to impairments in spatial memory (*Imayoshi et al., 2008*) and recognition memory (*Jessberger et al., 2009*).
- Positive behavioral effects of chronic antidepressants are associated with increased DG neurogenesis.

Analyses of Dentate Neurogenesis

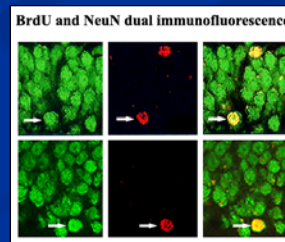


Kokaia and Lindvall, 2003

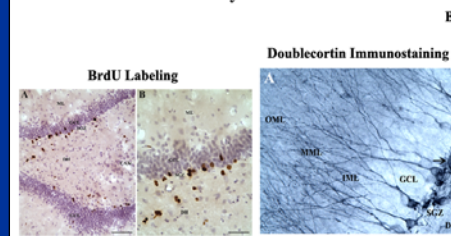
Neuronal Differentiation of Newly Born Cells



Differentiation of Newly Born Cells into Mature Neurons



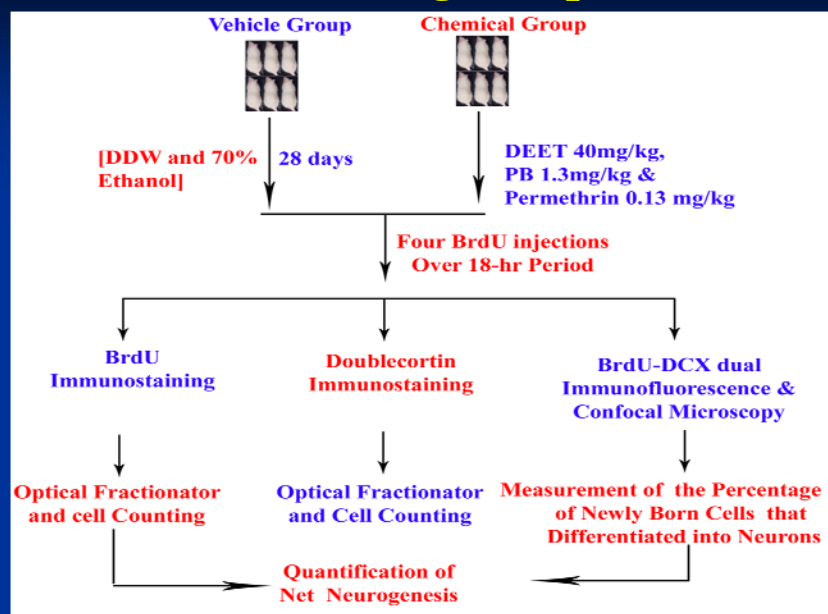
Identification of newly born cells and neurons



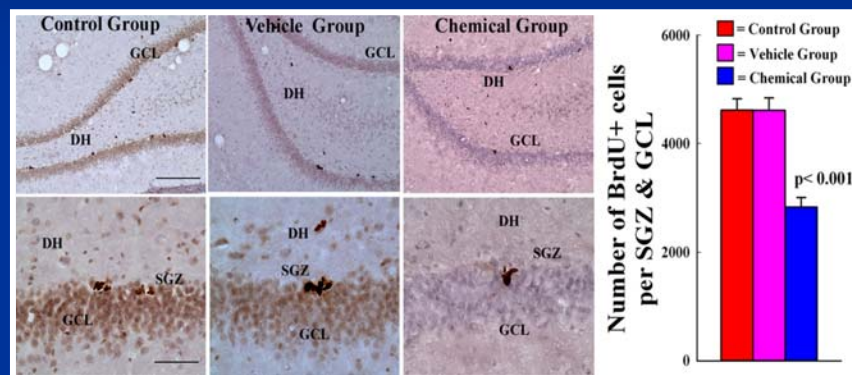
Rao & Shetty, *Eur. J. Neurosci.*, 2004

Rao et al., *Eur. J. Neurosci.*, 2005

Research Design (Expt. #1)

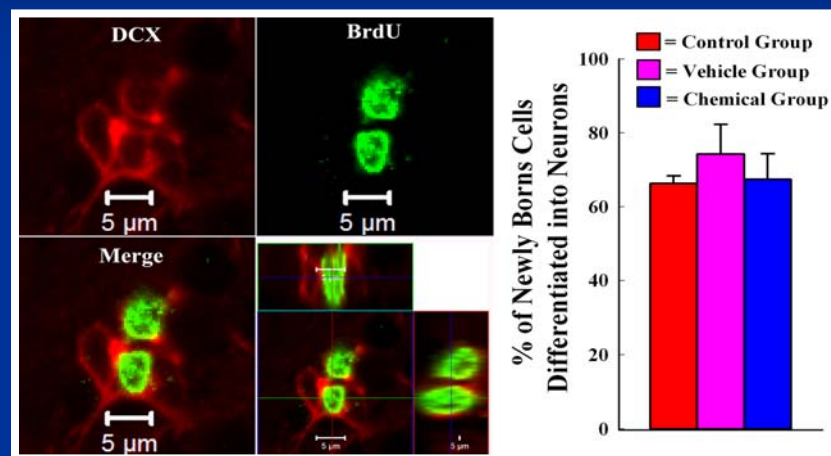


Short-term Effects of PB, DEET & Permethrin Exposure on Production of New Cells/Day in the Neurogenic Region (SGZ-GCL) of the Hippocampus

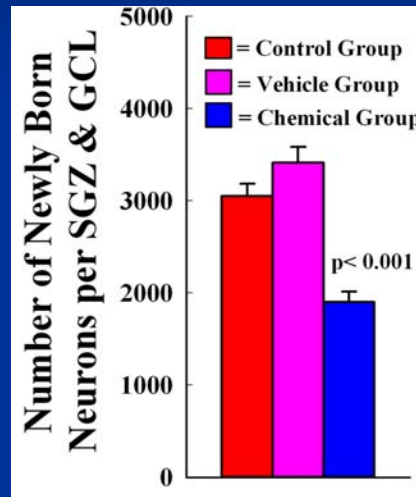


BrdU labeling Study (one injection every 6 hrs over an 18-hr period)

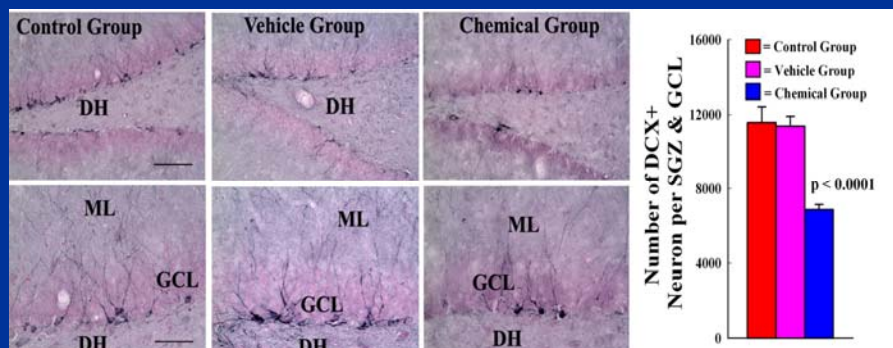
Short-term Effects of PB, DEET & Permethrin Exposure on Neuronal Fate-Choice Decision of Newly Born Cells in the Neurogenic Region of the Hippocampus



Short-term Effects of PB, DEET & Permethrin Exposure on Net Neurogenesis per Day in the Hippocampus



Short-term Effects of PB, DEET & Permethrin Exposure On The Status of Hippocampal Neurogenesis, as revealed by Doublecortin Immunostaining



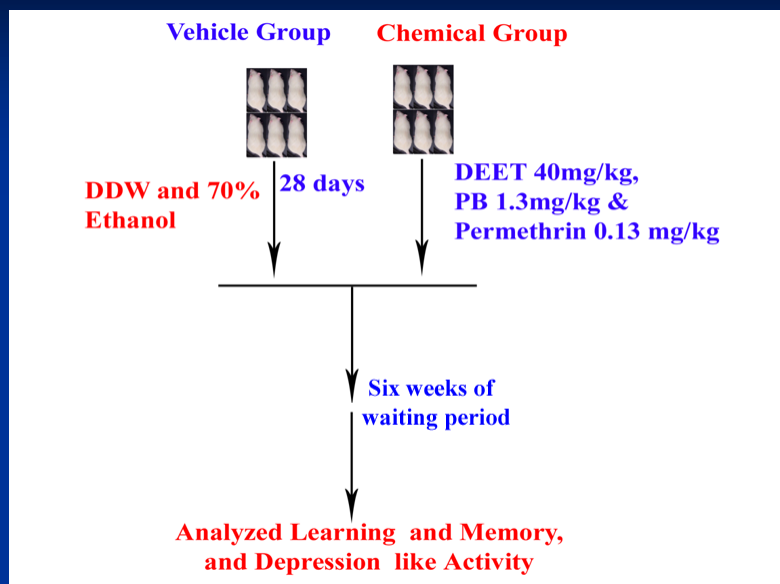
Conclusion (Expt. #1)

28-day exposure to a combination of GWI-related chemicals diminishes hippocampal neurogenesis in the immediate post-exposure period

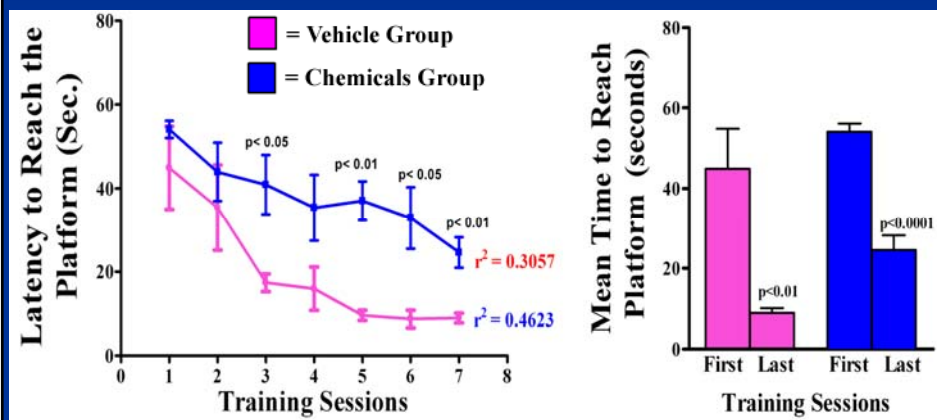
Expt. #2

Does the decline in hippocampal neurogenesis affect functions such as learning, memory and mood?

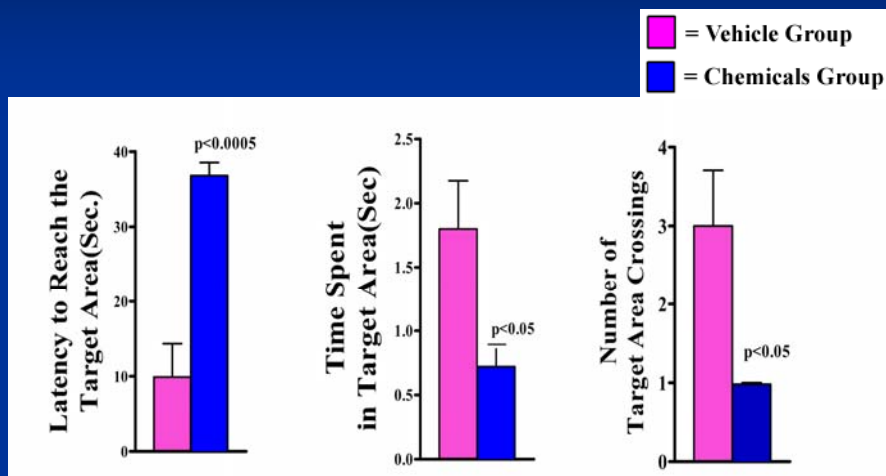
Research Design (Expt. #2)



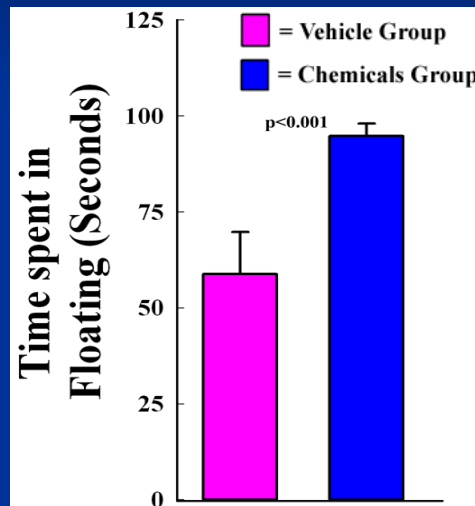
Effects of PB, DEET & Permethrin Exposure On Spatial Learning Function, as Examined by a Water Maze Test



Effects of PB, DEET & Permethrin Exposure On Memory Function, as Examined by a Memory Retrieval Test



Effects of PB, DEET & Permethrin Exposure On Depression, as Examined by a Forced Swim Test (FST)



Conclusions (Expt. #2)

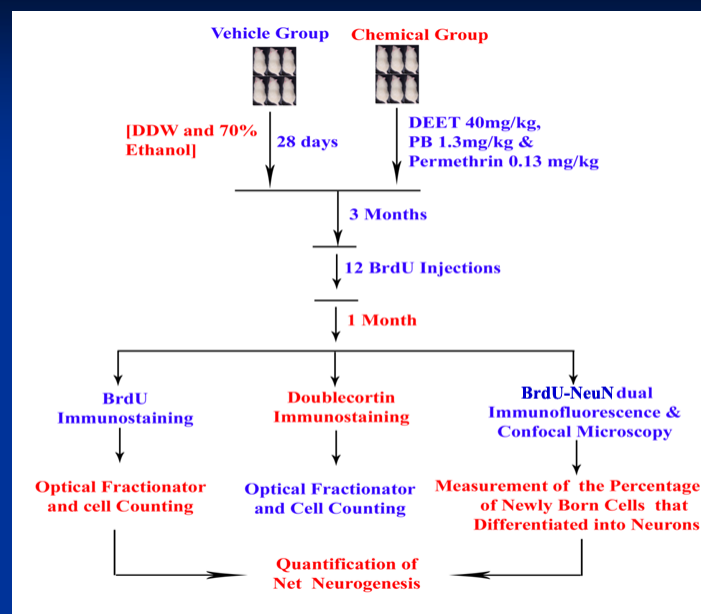
- (1) 28-day exposure to a combination of GWI-related chemicals leads to impairments in Functions such as learning, memory, and mood**
- (2) As learning, memory, and mood functions are linked to the extent of hippocampal neurogenesis, it is likely that declined hippocampal neurogenesis underlies cognitive dysfunction and depression in this model.**

Does the decline in hippocampal neurogenesis induced by the chemicals persists for prolonged periods after the exposure?

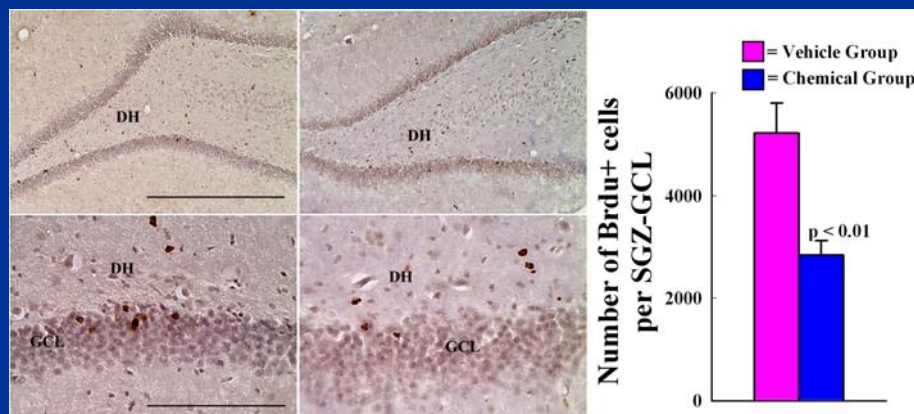
Expt. #3

Analyses of hippocampal neurogenesis at 3-months after the exposure regimen.

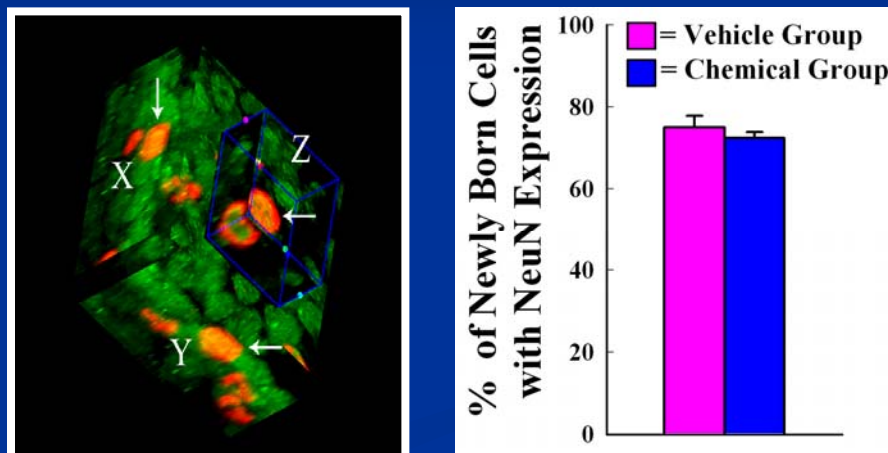
Research Design (Expt. #3)



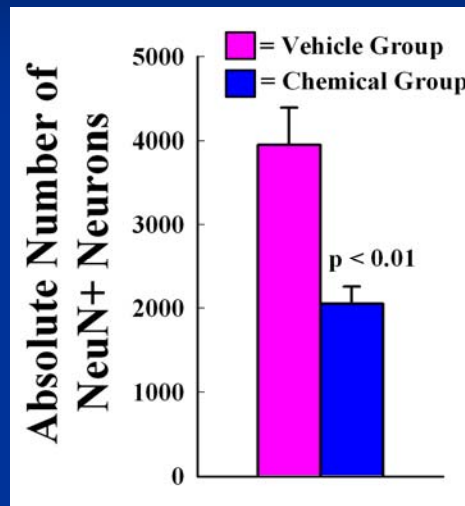
Long-Term Effects of PB, DEET & Permethrin Exposure on the Addition of New Cells to the Granule Cell Layer of the Hippocampus



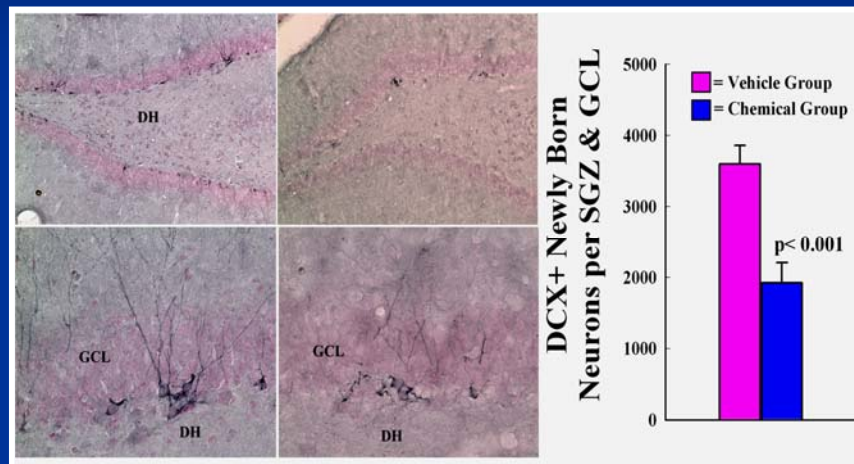
Long-Term Effects of PB, DEET & Permethrin Exposure on the Differentiation of Newly Born Cells into NeuN+ Mature Neurons in the Granule Cell Layer



Long-Term Effects of PB, DEET & Permethrin Exposure on Net Neurogenesis in the Hippocampus



Long-Term Effects of PB, DEET & Permethrin Exposure on the Status of Hippocampal Neurogenesis, as examined by Doublecortin Immunostaining



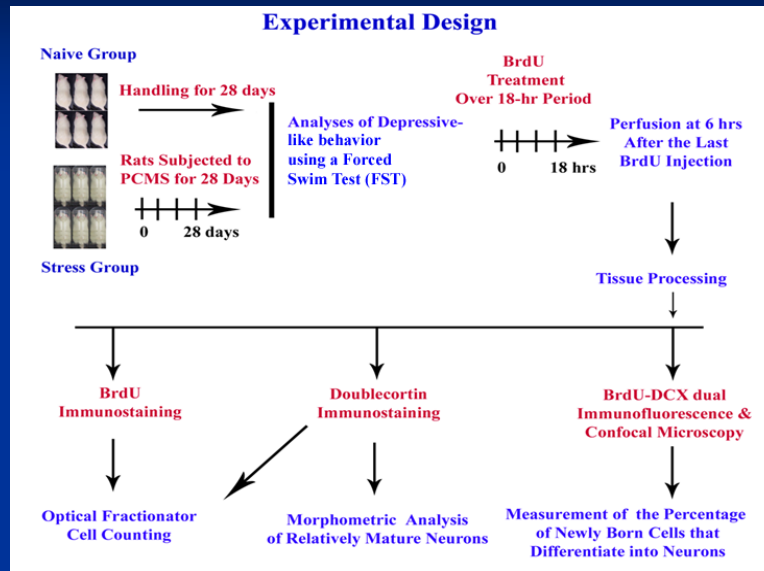
Conclusions (Experiments 1-3)

- 28-day exposure to a combination of GWI-related chemicals greatly diminishes hippocampal neurogenesis for prolonged periods.
- Reduced hippocampal neurogenesis is linked with impaired learning, memory and mood functions.
- Reduced hippocampal neurogenesis persists at four months after the exposure.

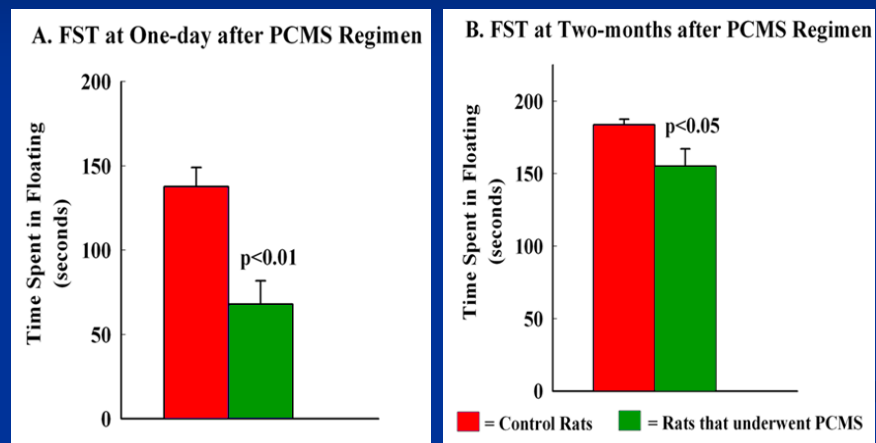
What happens if stress is added during the chemical exposure?

- Unpredictable Chronic Stress (UCS)
Well known to greatly increase stress hormones and decrease hippocampal neurogenesis and cause learning & memory impairments and depression.
- Predictable Chronic Mild Stress (PCMS)
5 minutes of restraint stress per day for 28 days

Effects of PCMS alone on Depression, and Hippocampal Neurogenesis (Expt. #4)

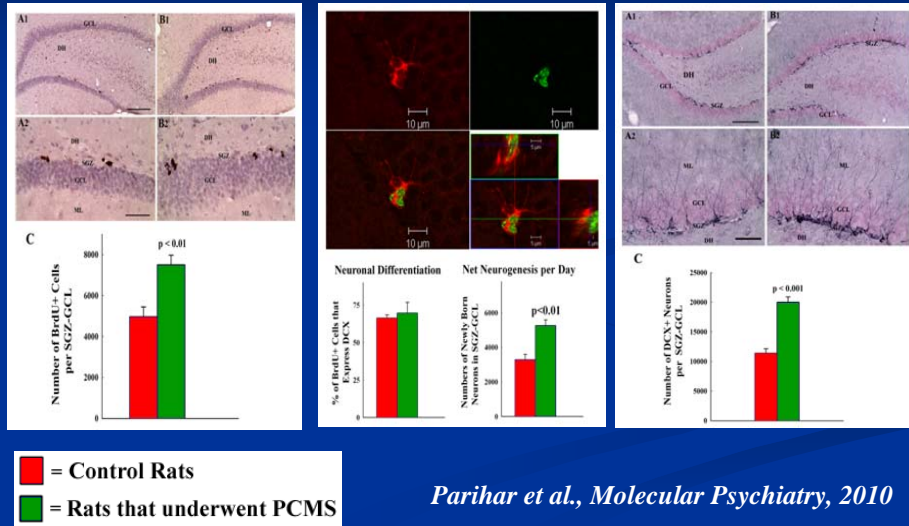


Effects of PCMS on Depressive-like Behavior in a Forced Swim Test (FST)



Parihar et al., Molecular Psychiatry, 2010

Effects of PCMS on the Extent of Hippocampal Neurogenesis



Effects of PCMS on Spatial Learning and Memory Function

Experimental Design

Naive Group



Handling for 28 days

Rats Subjected to PCMS for 28 Days

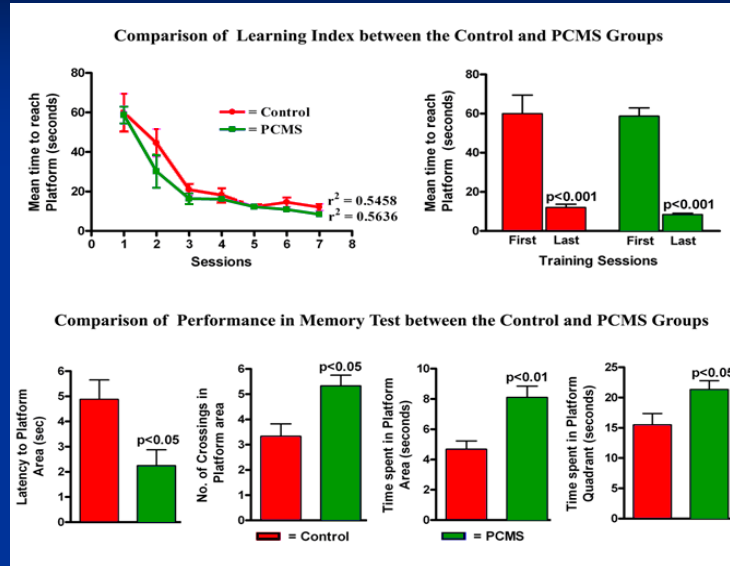
0 28 days

Spatial Learning & Memory Analyses at 1.5 months after PCMS Regimen

Novel Object Recognition Test (NORT)

Stress Group

PCMS Enhances Spatial Memory Retention Ability



PCMS Enhances Ability for Recognition Memory

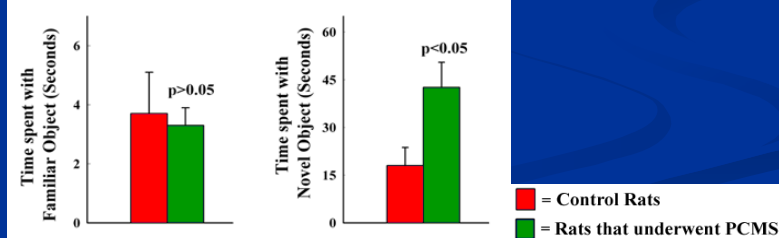
Exploration Phase



Testing Phase



Object Exploration in Memory Testing Phase of NORT



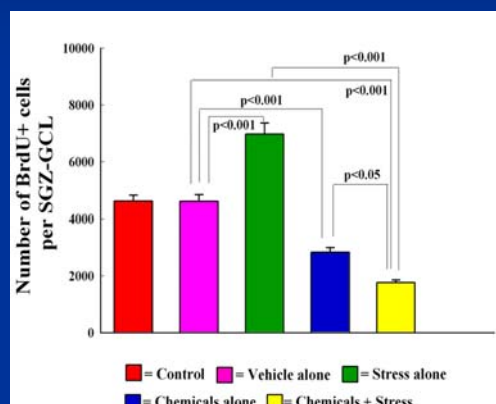
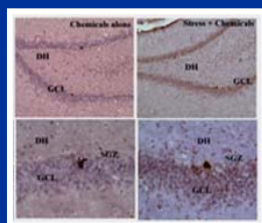
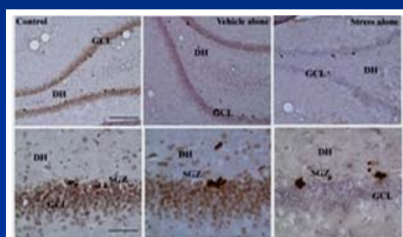
Conclusion (Expt. #4)

PCMS has beneficial effects, which include enhancements in hippocampal neurogenesis, mood and memory function.

Expt. #5

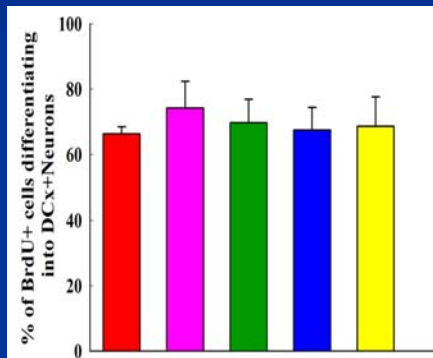
What happens if PCMS component is added during the exposure to three chemicals (PB, DEET, Permethrin)?

Short-term Effects of Combined Exposure to Chemicals and PCMS on the Production of New Cells/day in the Hippocampus

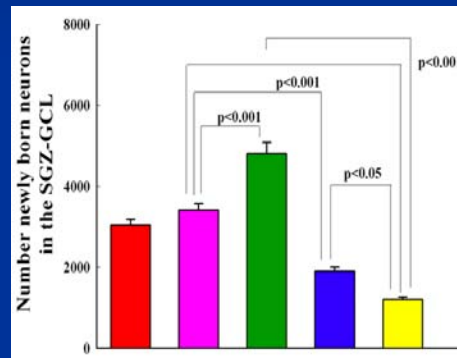


Short-term Effects of Combined Exposure to Chemicals & PCMS on: Neuronal Differentiation of Newly Born Cells & Net Neurogenesis

Neuronal differentiation of newly born cells

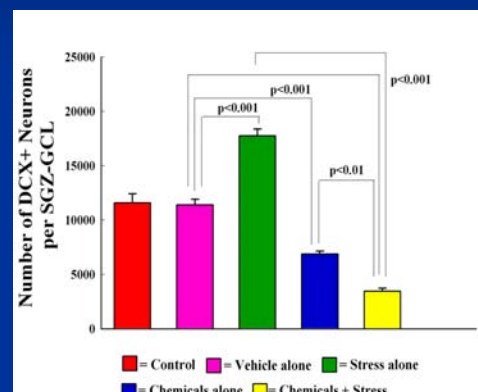
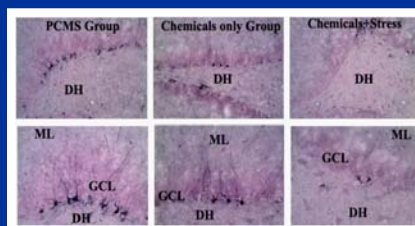
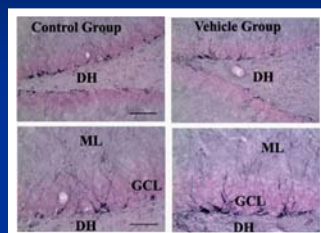


Net Neurogenesis



■ = Control ■ = Vehicle alone ■ = Stress alone
■ = Chemicals alone ■ = Chemicals + Stress

Short-term Effects of Combined Exposure to Chemicals and PCMS on the Status of Hippocampal Neurogenesis



■ = Control ■ = Vehicle alone ■ = Stress alone
■ = Chemicals alone ■ = Chemicals + Stress

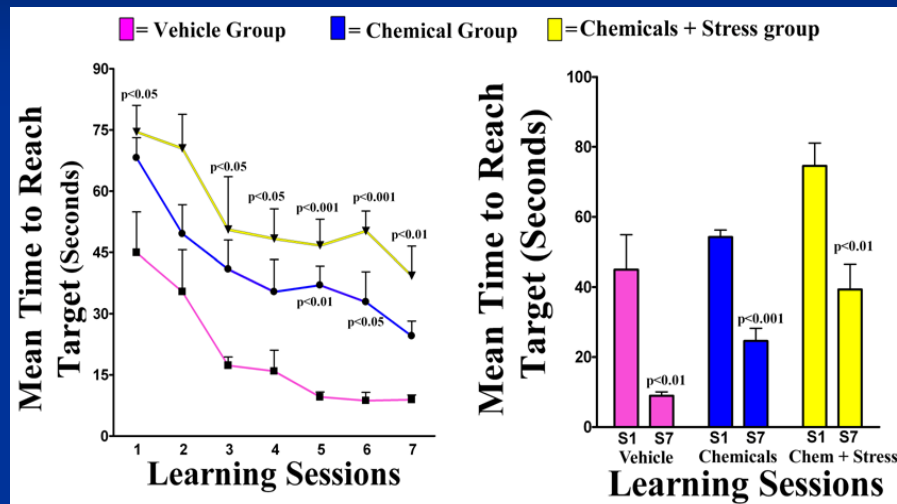
Conclusion (Expt. #5)

Addition of mild stress (PCMS) exacerbates the effects of GWI-related chemicals on hippocampal Neurogenesis.

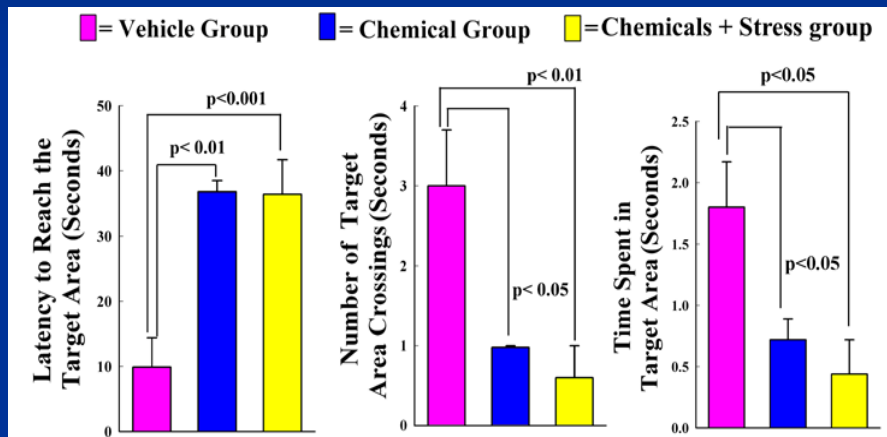
Expt. #6

Long-term effects of combined exposure to GWI-related chemicals & PCMS on learning, memory and mood functions and hippocampal neurogenesis.

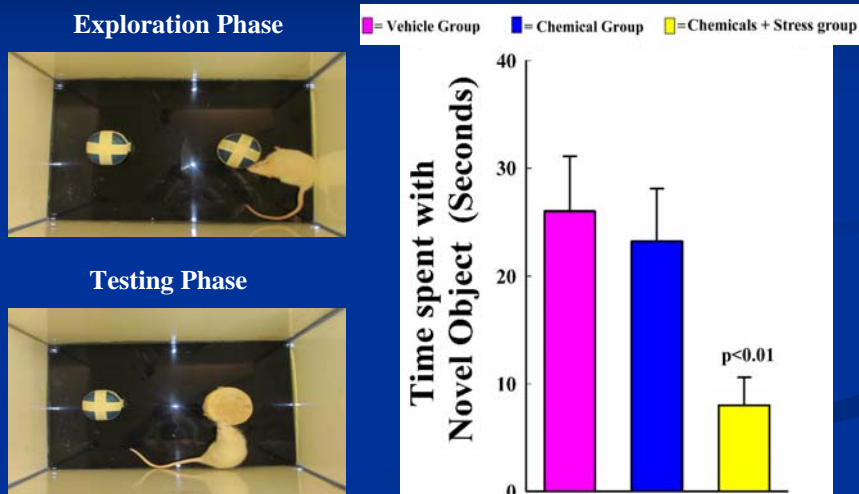
Effects of combined exposure to GWI chemicals & PCMS on spatial learning function, as examined by a water maze test



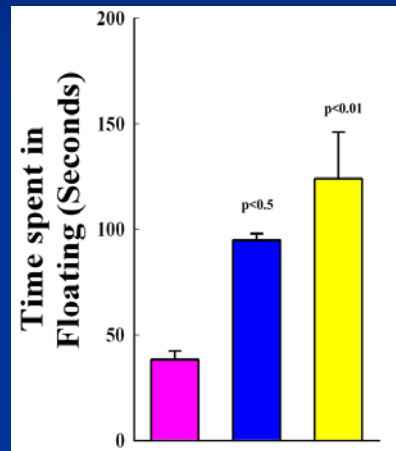
Effects of combined exposure to GWI chemicals & PCMS on memory, as examined by a memory retrieval test



A Combined Exposure to Chemicals & PCMS Impairs Recognition Memory



Effects of combined exposure to GWI chemicals & PCMS on depression, as examined by a forced swim test (FST)



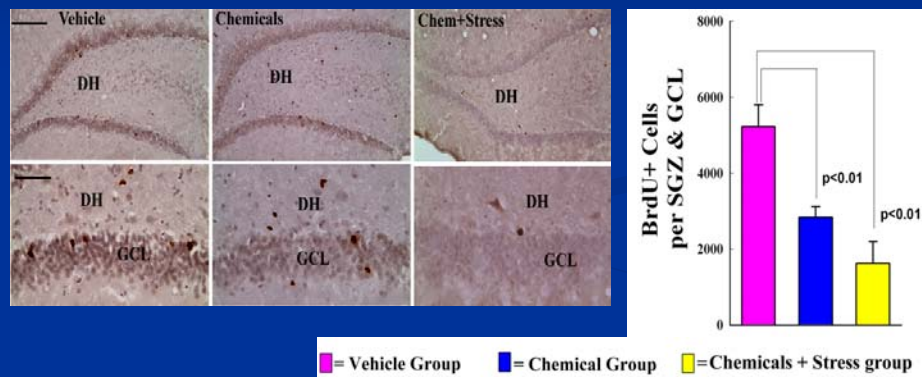
■ = Vehicle Group ■ = Chemical Group ■ = Chemicals + Stress group

Conclusions (Expt. #6)

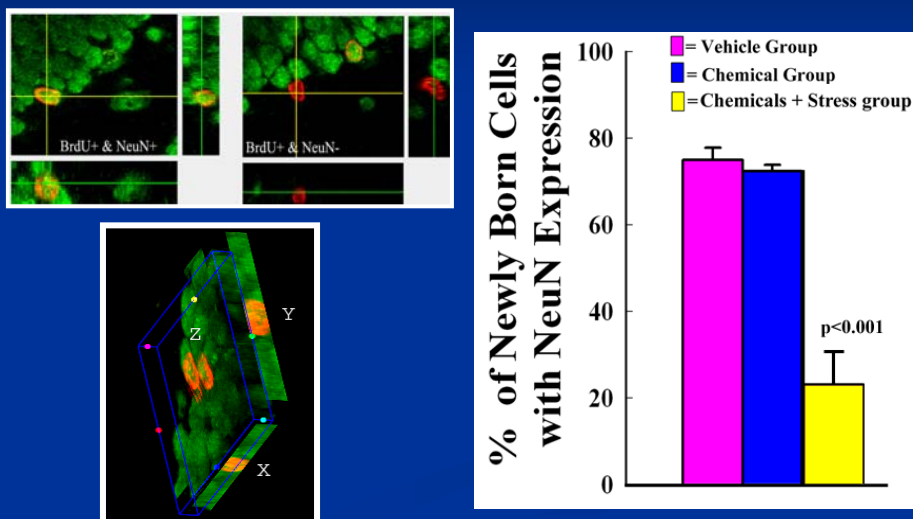
Addition of mild stress (PCMS) increases the overall adverse effects of GWI-related chemicals on functions such as learning, memory & mood.

As learning, memory, and mood functions are linked to the extent of hippocampal Neurogenesis, it is likely that decline in hippocampal neurogenesis underlies cognitive dysfunction and depression in this model.

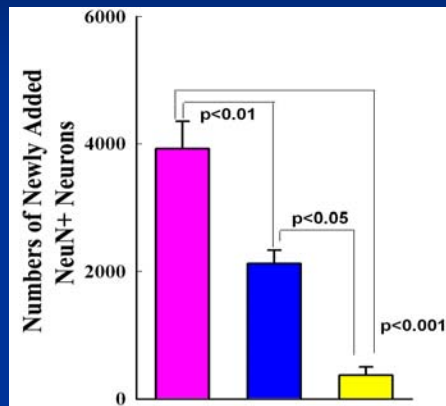
Long-term effects of combined exposure to GWI-related chemicals & PCMS on the addition of new cells to the hippocampus



Long-term effects of combined exposure to GWI-related chemicals & PCMS on the differentiation of newly born cells into NeuN+ mature neurons



Long-term effects of Combined Exposure to GWI-related chemicals & PCMS on Net Neurogenesis in the Hippocampus



■ = Vehicle Group ■ = Chemical Group ■ = Chemicals + Stress group

Conclusions (Expt . #6)

- *Addition of mild stress (PCMS) during the exposure to GWI-related chemicals enhances the adverse long-term effects of these chemicals on hippocampal neurogenesis.*
- *Enhanced adverse effect on neurogenesis is associated with worsening of functions such as learning, memory and mood.*
- *Thus, stem cell dysfunction in the hippocampus likely underlies the cognitive and mood impairments observed in this GWI model.*

Is the adverse effect of GWI-related chemicals specific to hippocampal stem cells?

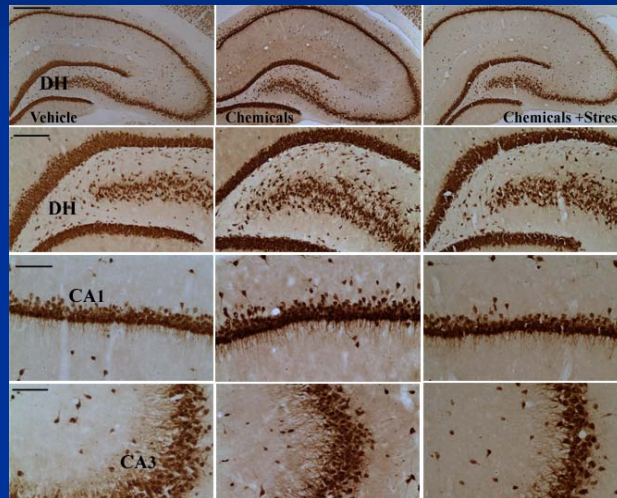
(1) Possible loss of neurons in different regions of the hippocampus.

Distribution of NeuN+ neurons in the DG, CA1 & CA3 subfields at 4 months after the exposure.

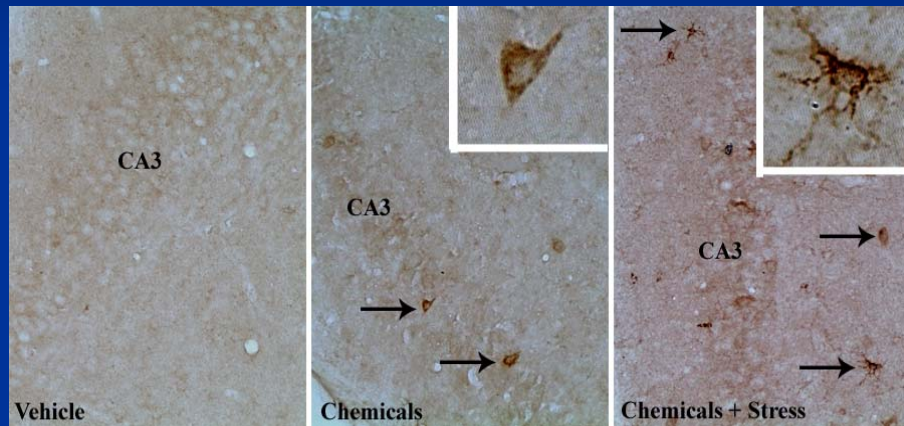
(2) Inflammation in the hippocampus.

Analyses of activated microglia using ED-1 immunostaining.

Exposure to GWI-related chemicals or GWI chemicals & stress does not induce widespread hippocampal neurodegeneration

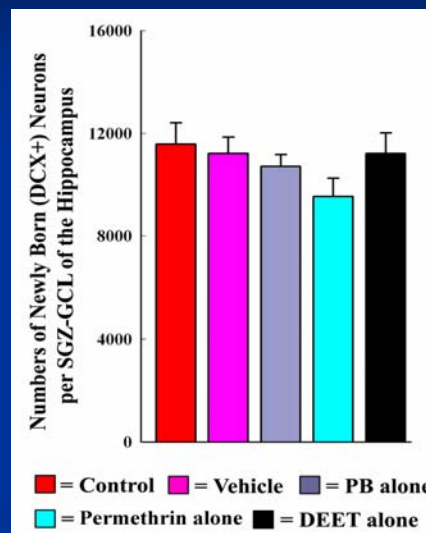
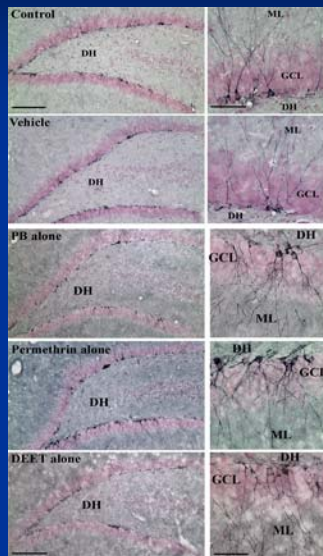


Exposure to GWI-related chemicals or GWI chemicals & stress does induce some inflammation in the hippocampus



Activated microglia – ED1 Immunohistochemistry

Exposure to a single GWI-related chemical (PB, permethrin or DEET) does not decrease hippocampal neurogenesis



Overall Conclusions

1. A combined exposure to GWI-related chemicals **impairs hippocampal neurogenesis as well as hippocampal-dependent functions such as learning, memory and mood.**
2. **The adverse effects appear to be due to an interaction of the three chemicals,** as exposure to any of these chemicals alone has no significant effect on neurogenesis.
3. **Exposure to a combination of the GWI-related chemicals appears to have a specific effect on hippocampal stem cell function,** as this exposure did not induce widespread hippocampal neurodegeneration or inflammation.
4. **Presence of even a mild stress during the exposure exacerbates the various adverse effects of GWI-related chemicals.**

ACKNOWLEDGMENTS

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