Modeling the Genetic Basis of Individual Differences in Susceptibility to Gulf War Illness

Pre-Clinical Animal Studies

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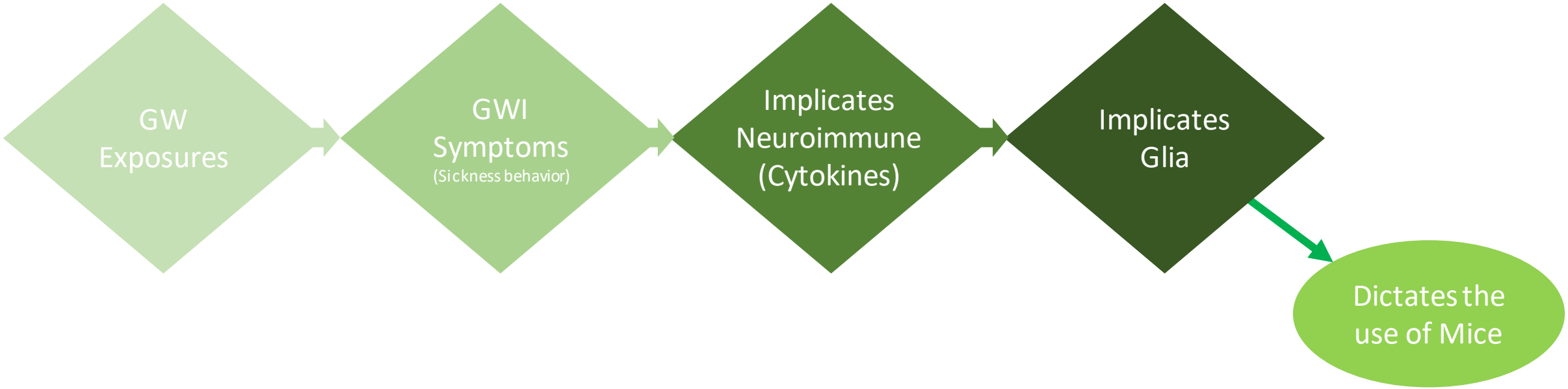
Considerations for development of an animal model of GWI



1. Evaluate potential Basis of GWI: Brain Immune Interactions
2. Study the relevant cell types: glia
3. Take advantage of glial specific reporter mice and different strains

Animal models allow investigation of initiating events and progression of Gulf War Illness

Mouse models are being used to:

1) Study exposures and conditions that model those that occurred in the Gulf War:
   - Organophosphates (**DFP**, CPF, DDVP, sarin)
   - Prophylactic against nerve agent exposure (PB)
   - Stressor surrogate (stress hormone, corticosterone (CORT))

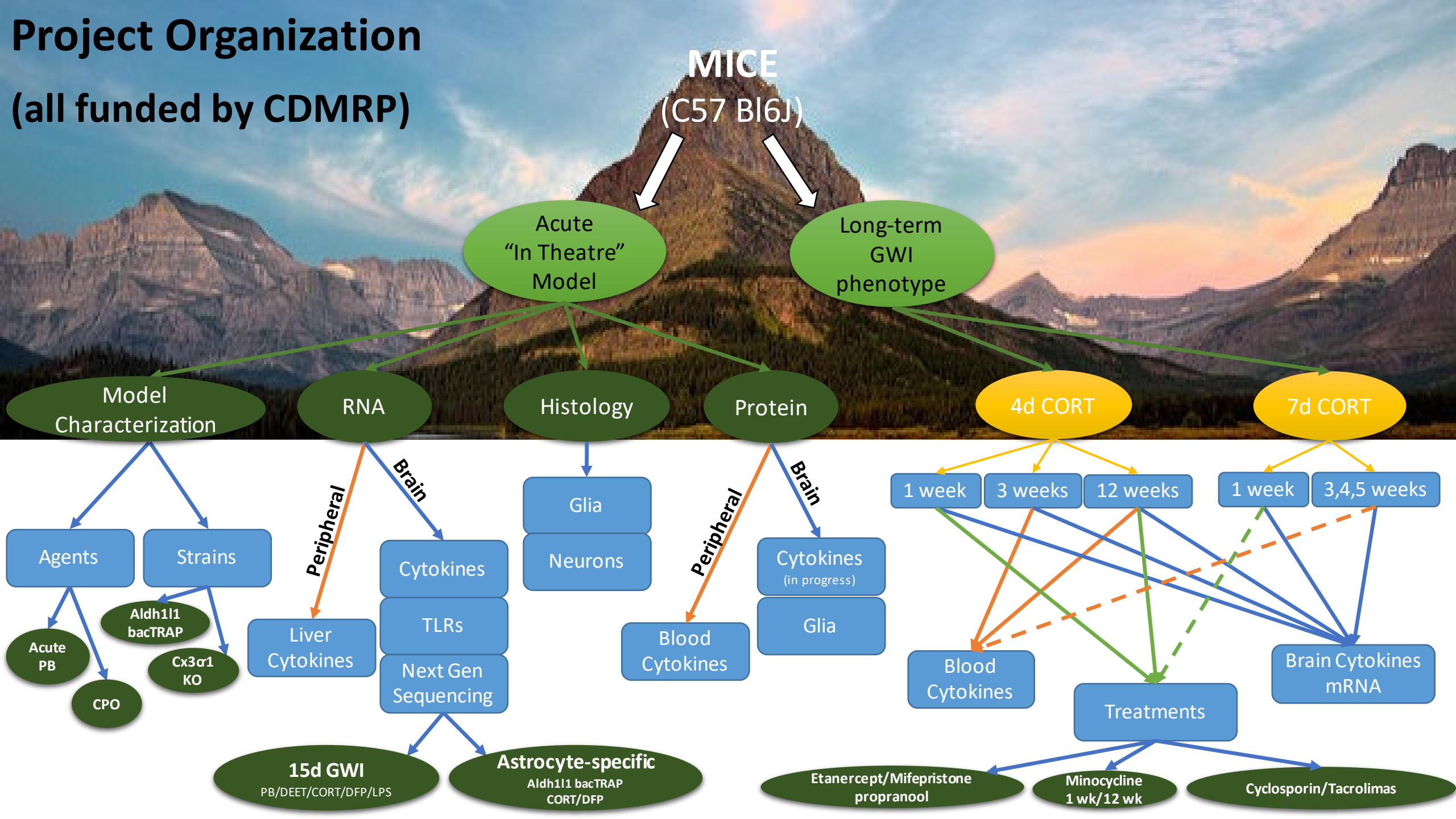
2) Evaluate endpoints (initial and long term) that underlie symptoms in ill veterans:
   - Brain cytokines and chemokines that cause sickness behavior

3) Model time-course of GWI:
   - Demonstrate effects at outset of exposure and that persist for over 30 years

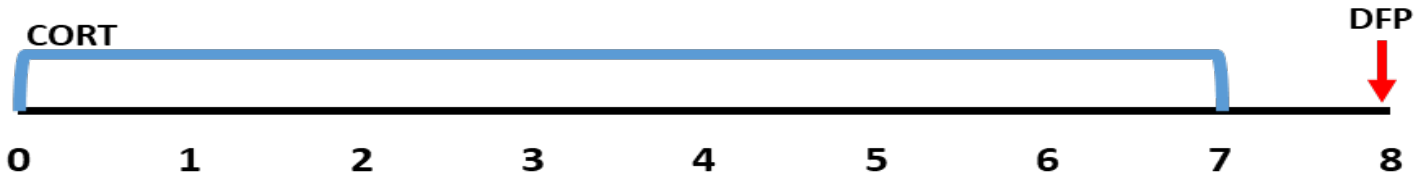
4) Evaluate potential therapies:
   - Show effectiveness of candidate therapies to alter brain cytokines/chemokines in short term GWI phenotype screening studies.
   - Administer best treatments at GWI relevant long term time points.

Project Organization

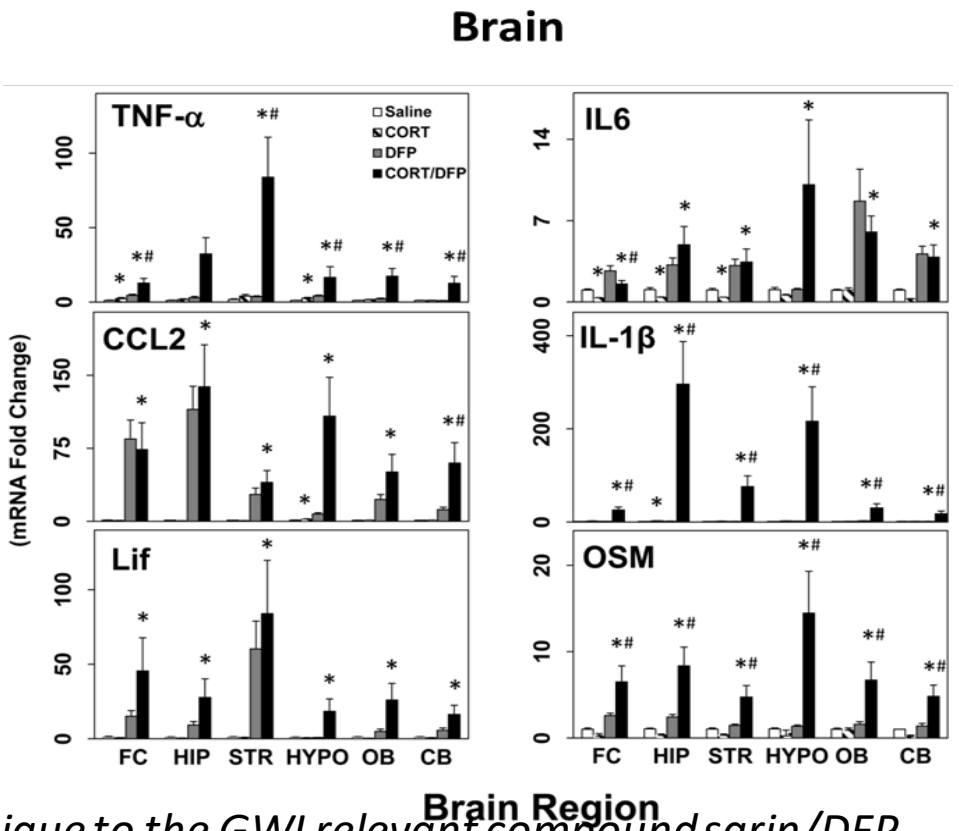
(all funded by CDMRP)



ACUTE NEUROINFLAMMATION MODEL OF GWI



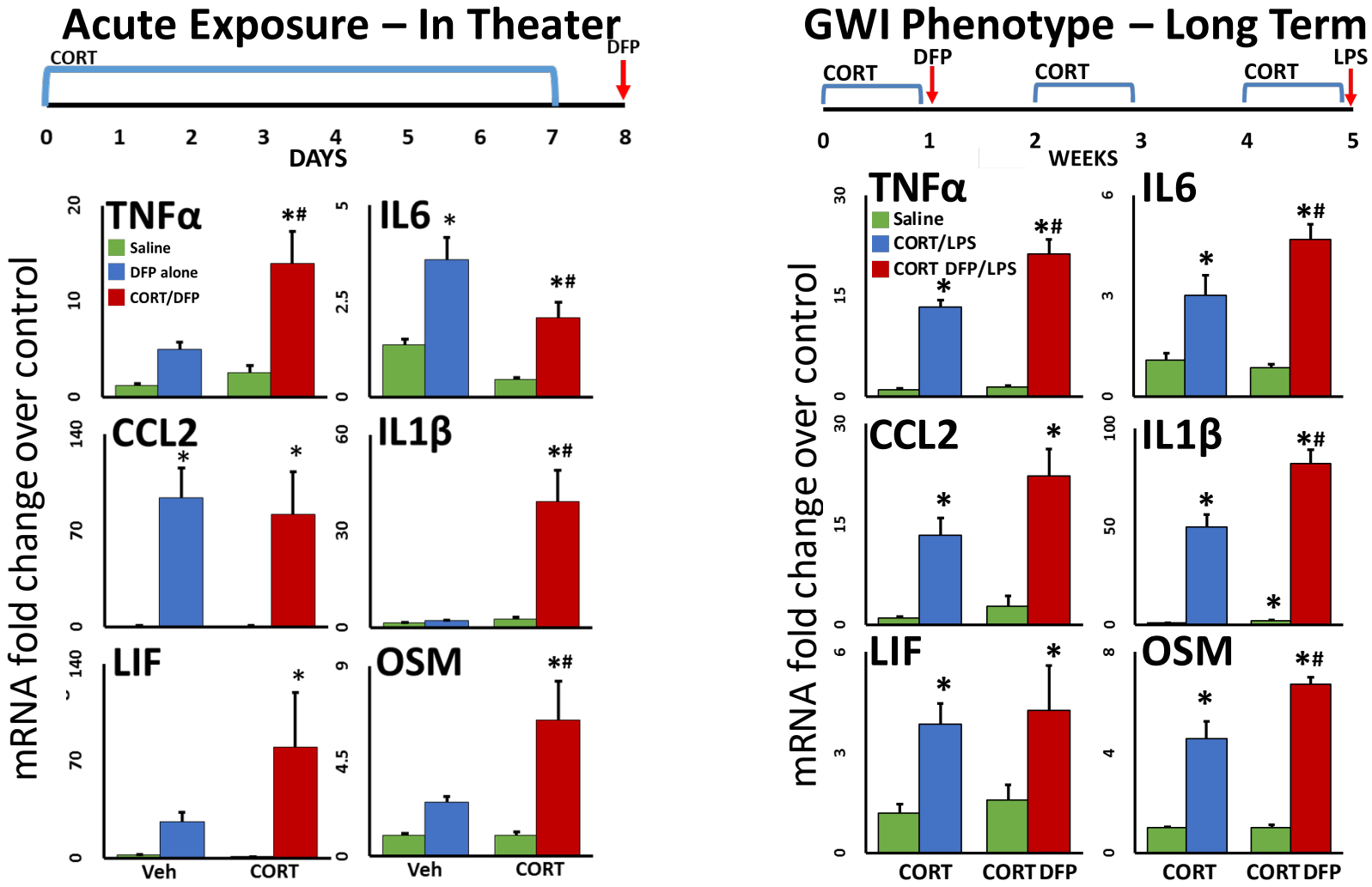
- Diisopropyl fluorophosphate (DFP), used as sarin surrogate - unexpectedly causes brain "neuroinflammation"; effects consistent with "sickness" behavior in a mouse model.
- The anti-inflammatory rodent stress hormone, corticosterone (CORT), even more unexpectedly, makes DFP neuroinflammation markedly worse
- Taken together, these observations led to a neuroinflammation model of GWI based on combined exposure to physiological stress and nerve agent



This neuroinflammatory effect may not be unique to the GWI relevant compound sarin/DFP alone but other OPs and conditions soldiers were exposed to during service

Developing a Mouse Long-term Model of GWI

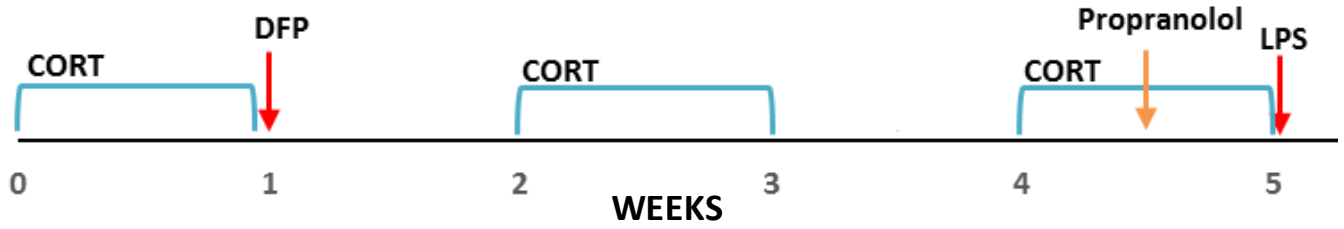
- Exposure to CORT DFP not only instigates acute neuroinflammation, but also affects the long-term response to future immune challenge



p ≤ 0.05 compared to appropriate control; between groups (Veh vs CORT; CORT vs CORT DFP*

Propranolol as a candidate GWI therapy in long-term model

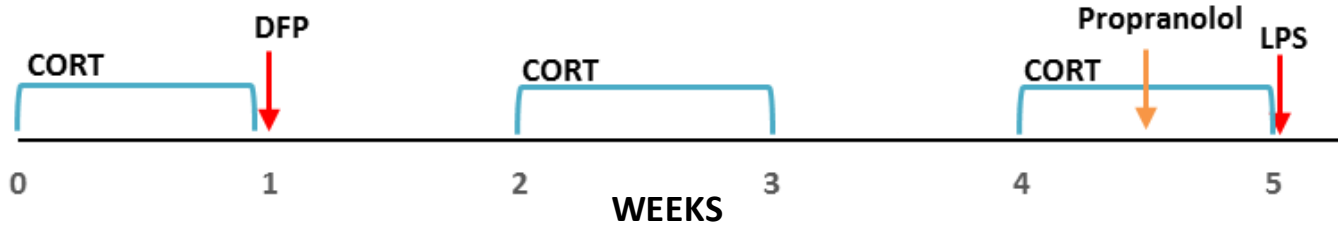
- Propranolol is a beta-blocker that has been shown to have anti-inflammatory properties

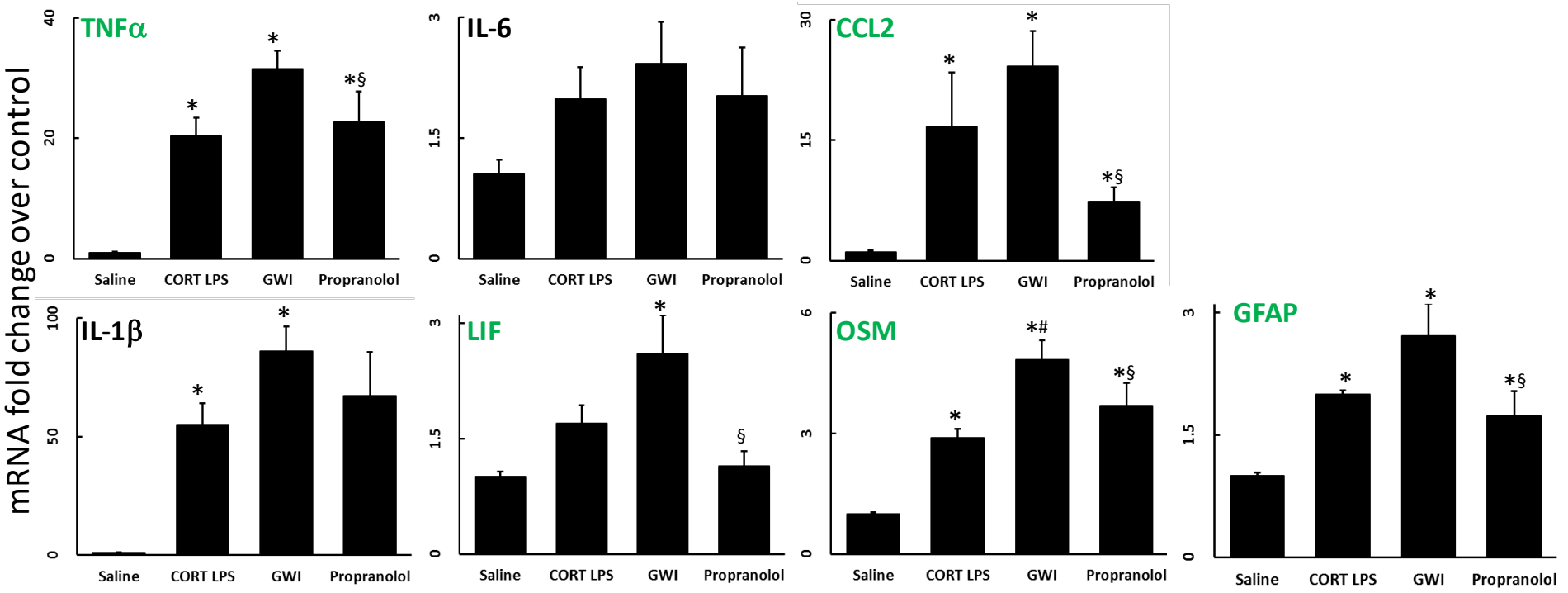


- The goal of our treatment strategy has been to treat the underlying cause of GWI, not to identify more drugs that may ameliorate specific symptoms or flare-ups

- We also don't want to abolish healthy immune responses, so we use CORT LPS (all conditions except the GW-relevant toxicant [DFP] exposure) as a "healthy control" immune response
  - Everyone experiences periods of physiological stress and immune challenge (i.e. injury, viral or bacterial infections, etc)

Propranolol reduces neuroinflammation associated with GWI





p ≤ 0.05: compared to saline; compared to CORT LPS#; compared to GWI§*

Conclusions

- We developed “in theater” and long-term GWI animal models largely based on C57Bl6 male mice but also extended to rats.

- The stressor (CORT)-enhanced neuroinflammation phenotype was extended beyond exposure to the sarin surrogate DFP, as chlorpyrifos, dichlorvos and sarin exposures all caused the same phenotype.

- Acetylcholinesterase was not implicated as a target for these effects suggesting that other targets remain to be identified.

- Propranolol (and other drugs) ameliorated the CORT-DFP neuroinflammation in the long-term model.

Lab Chiefs

- Jim O'Callaghan
- Diane Miller

Postdocs-Lieutenants

- Kimberly Kelly
- Alicia Locker
- Lindsay Michalovicz
- Julie Vrana

Lab Technicians

- Chris Felton
- Brenda Billig
- Fang Ma
- Ali Yilmaz

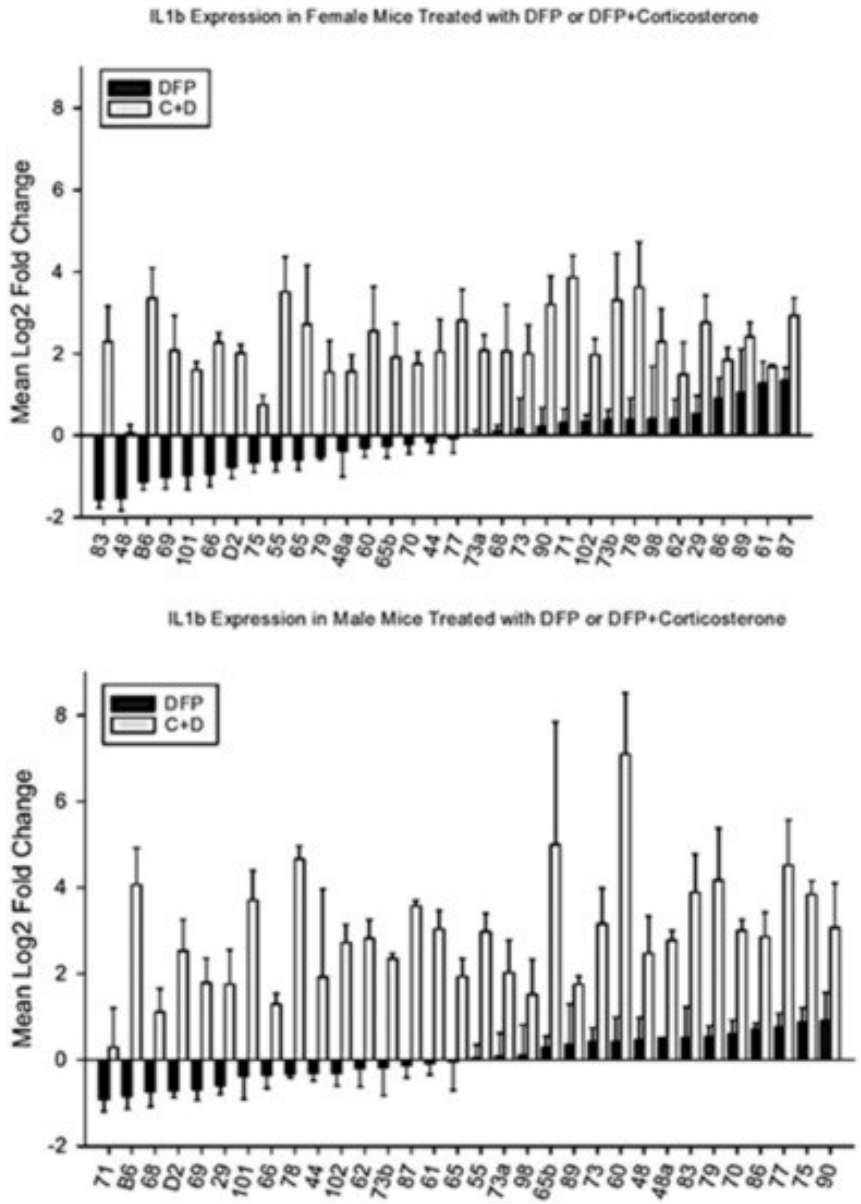
Part 2 – Genetics and Genomics

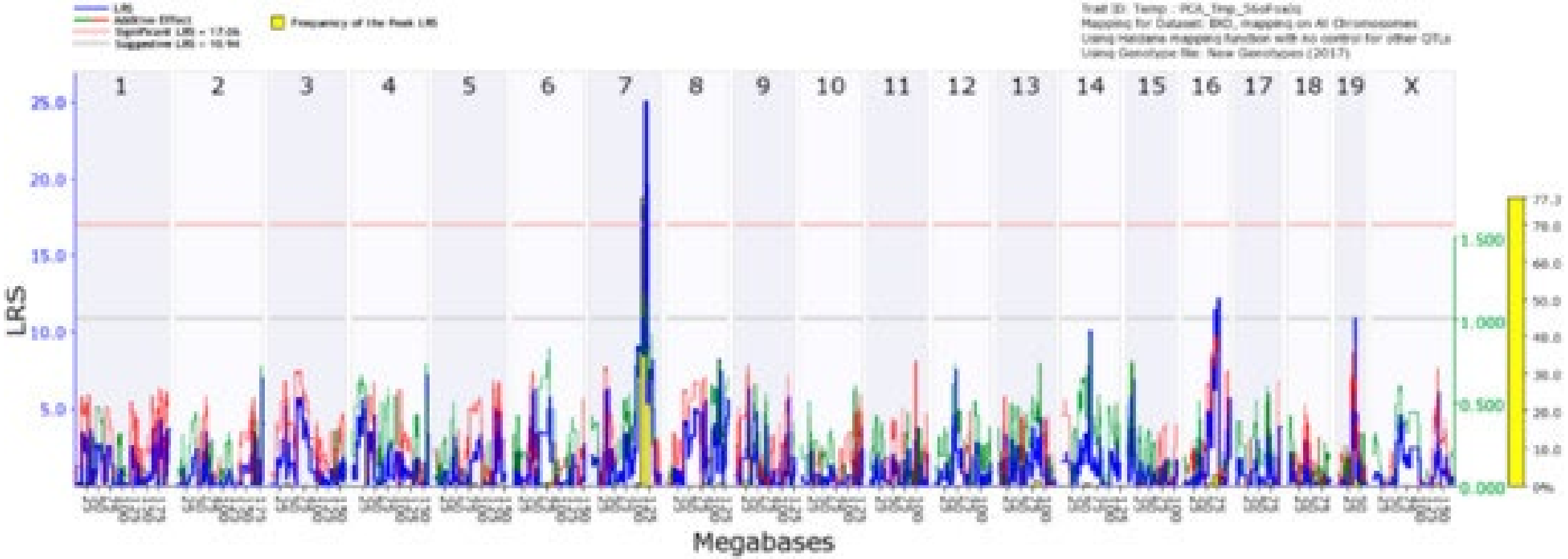
- At a Society of Toxicology meeting, I saw Jim's presentation and was impressed that the model involved the C57BL/6J mouse. I then proposed that we investigate possible genetic differences in susceptibility by testing another mouse strain, DBA/2J.

What about susceptibility?

- Recall that 25-30% of GW veterans became ill
- What about those who did not, all else being equal?
- Can a mouse model address this?
- O'Callaghan and Miller developed the exposome model using the C57BL/6J (B6) mouse.
- The B6 strain is one of two founders of a large panel of recombinant inbred mouse strains – the other founder is DBA/2J (D2). The Panel is called BXD and contains 150 such strains.
- Preliminary work showed the D2 strain to be much less sensitive to the OP+CORT treatment. Also, females of both strains were less sensitive to the treatment.
- Accordingly, we expanded the experiment to 30 BXD

Here, we show he effect of DFP and DFP+CORT on expression of *Il1b* in prefrontal cortex in 30 BXD recombinant inbred mouse strains. The X-axis lists the strains by their numerical identifier and the Y-axis presents Log_2 expression of *Il1b.* The top panel presents female data and bottom panel, male data. These results support the observation by O'Callaghan and Miller that CORT greatly increases expression of this gene by DFP. Overall, females were less affected by CORT+DFP than males



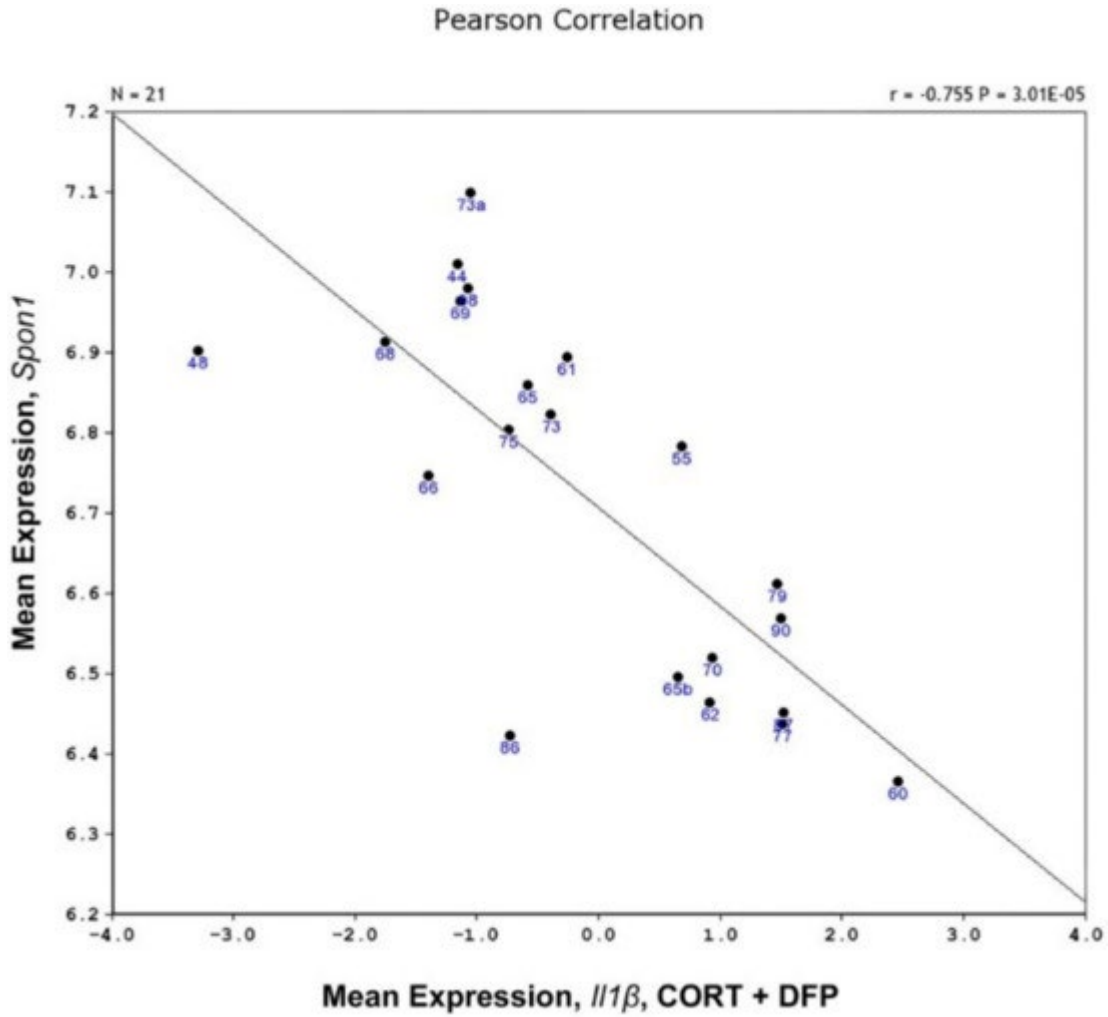


Mapping the phenotype shown in the previous slide—female and male data combined. There is a highly significant association between a marker (snp) and expression of *Il1b* on chromosome 7. Are there any possible candidate genes near the marker?

One possible candidate gene located near the marker is *Spon1* that codes for the protein, spondin or f-spondin in humans. It is *cis*-regulated and highly correlated with our aggregate phenotype as illustrated in the scatterplot.

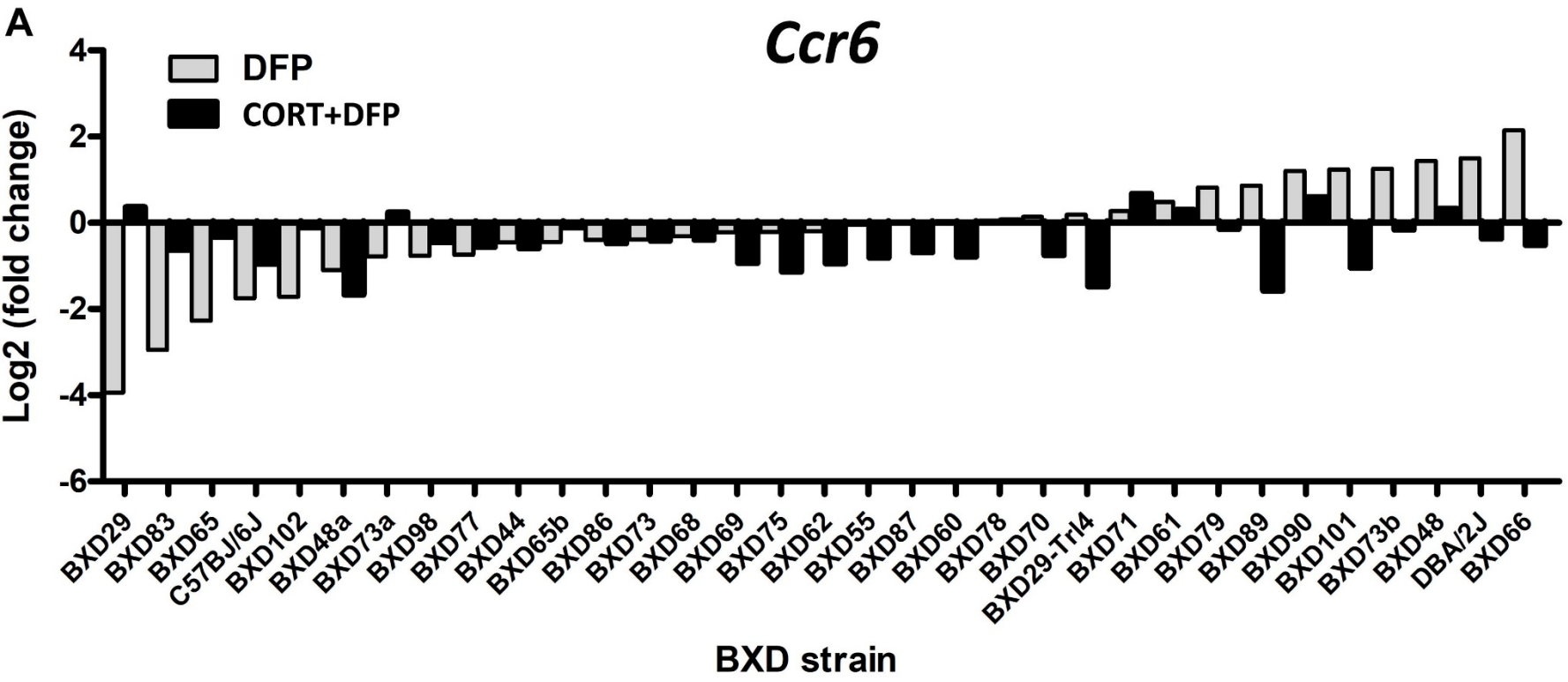
What does spondin do?

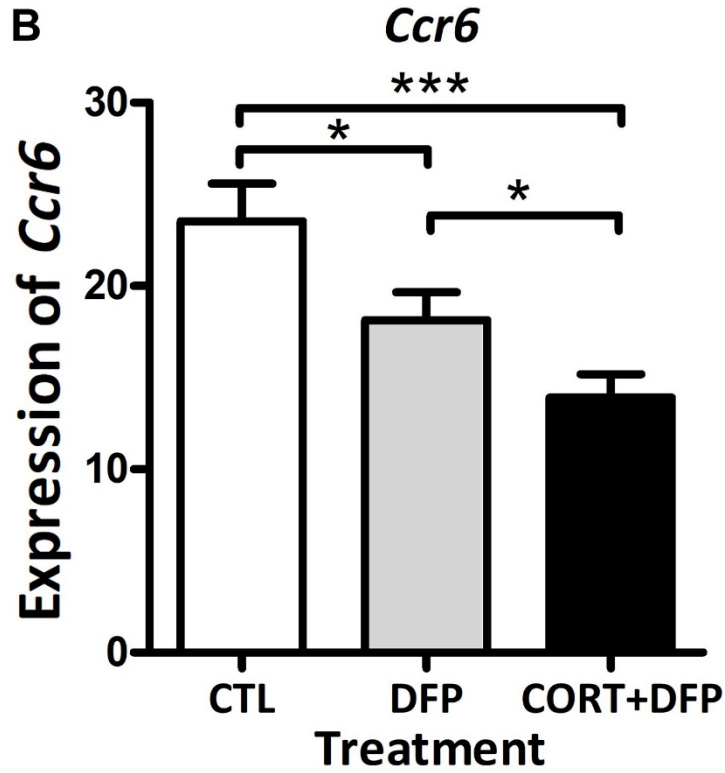
Organizes basal plate in development
Axon guidance
Activates Wnt signalling
Marker for AD dementia
Inhibits Aβ



What about genomic markers?

- We subjected prefrontal cortex tissue from animals treated with CORT+DFP to RNA-seq.

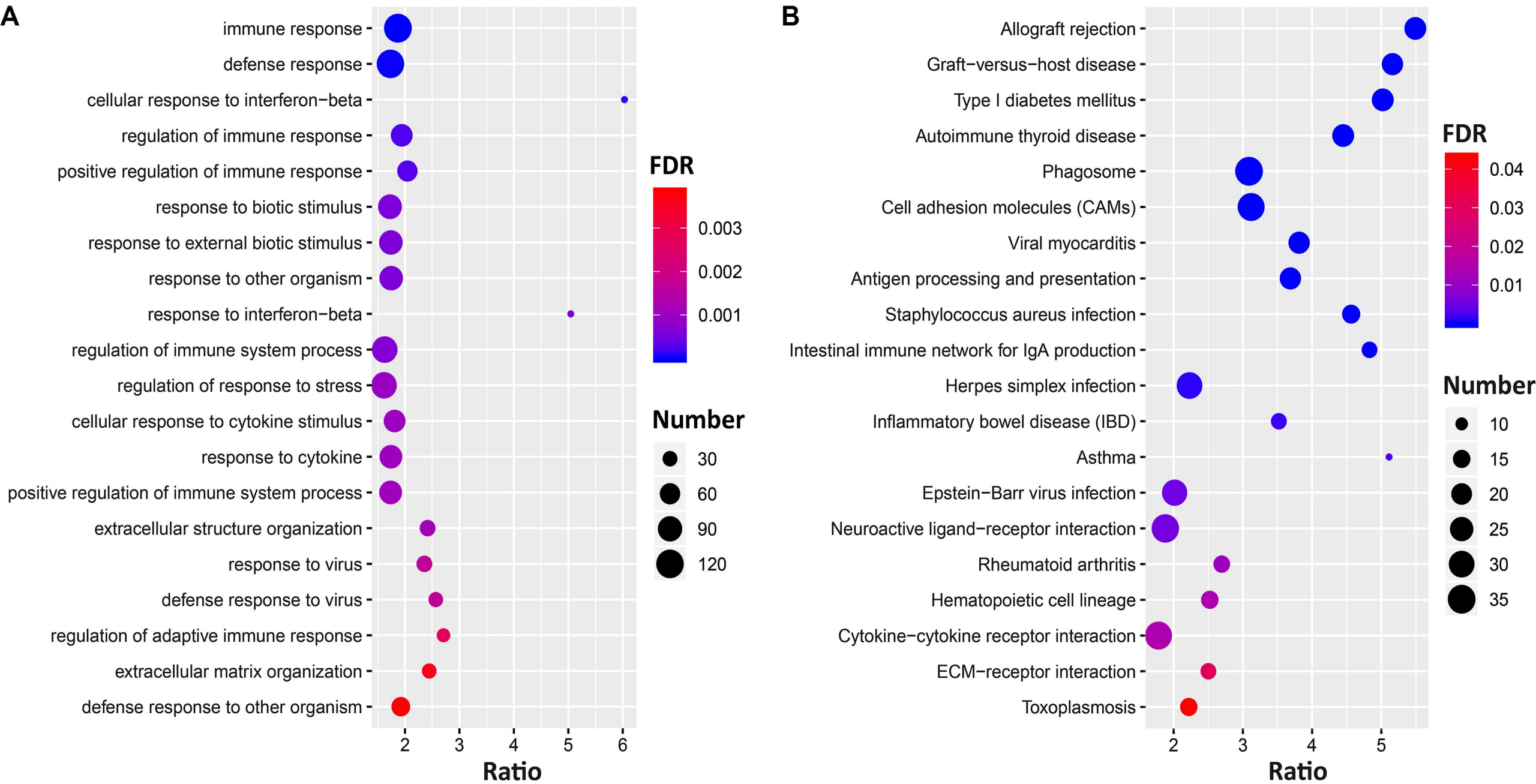




The candidate gene identified here was chemokine receptor 6 or *Ccr6 in the* Ccr6-CCL20 pathway. The effects of DFP and CORT+DFP are seen in the right panel and the strain distribution in the left.

What does Ccr6 do?

- major role in driving T-helper differentiation in inflammatory diseases and maintaining leukocyte homeostasis
- regulates the migration of inflammatory and regulatory T cells
- Cytokine-cytokine interactions
- Possible role in autoimmune disease like ALS



Gene ontology analysis (left panel) Kyoto Encyclopedia of genes and genomes (right panel) among CORT+DFP groups

Conclusions

- Highly significant gene X environment effects in mouse model of GWI
- Significant sex differences
- Identification of phenotypic and expression-based candidate genes
- May produce new targets for therapeutics, prophylaxis
- Sets the stage for new study of epigenetics – relative to the chronic nature of the disease.

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