



Does Early Life Inflammation Contribute to the Risk for  
Developing Psychiatric Conditions?

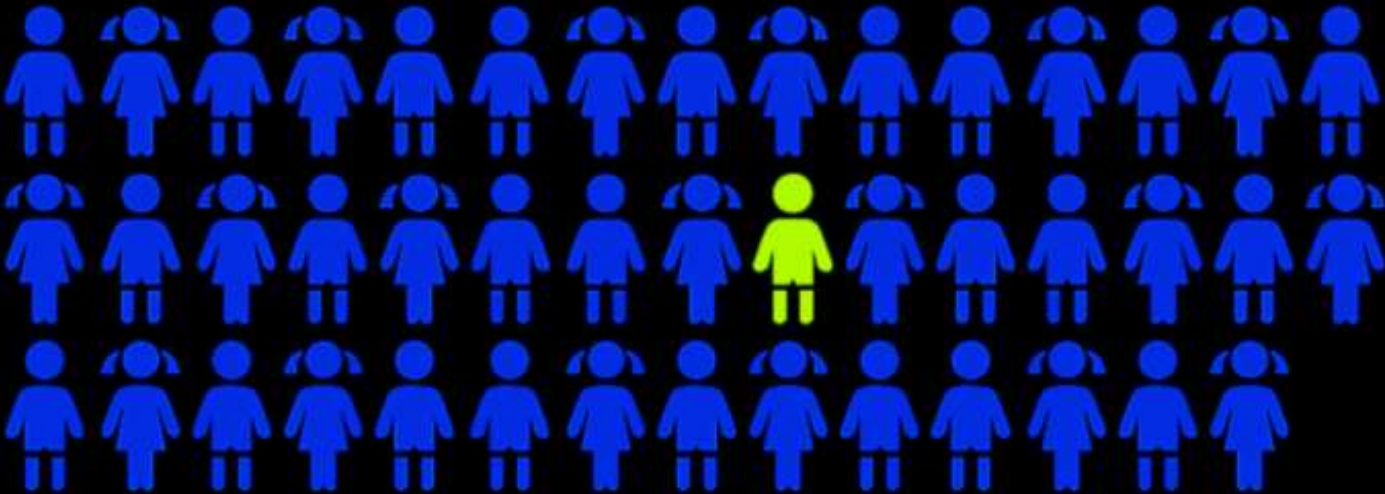
Galen Missig, PhD

6.14.22

# Disclosures

- As of December 2021 I am employed as a Senior Scientist at Cerevel Therapeutics, LLC.
  - This presentation is from prior work and does not represent the views of Cerevel Therapeutics, LLC.
- The work described here was done while I was a researcher at McLean Hospital which included support from the BBRF (Young Investigator Award)

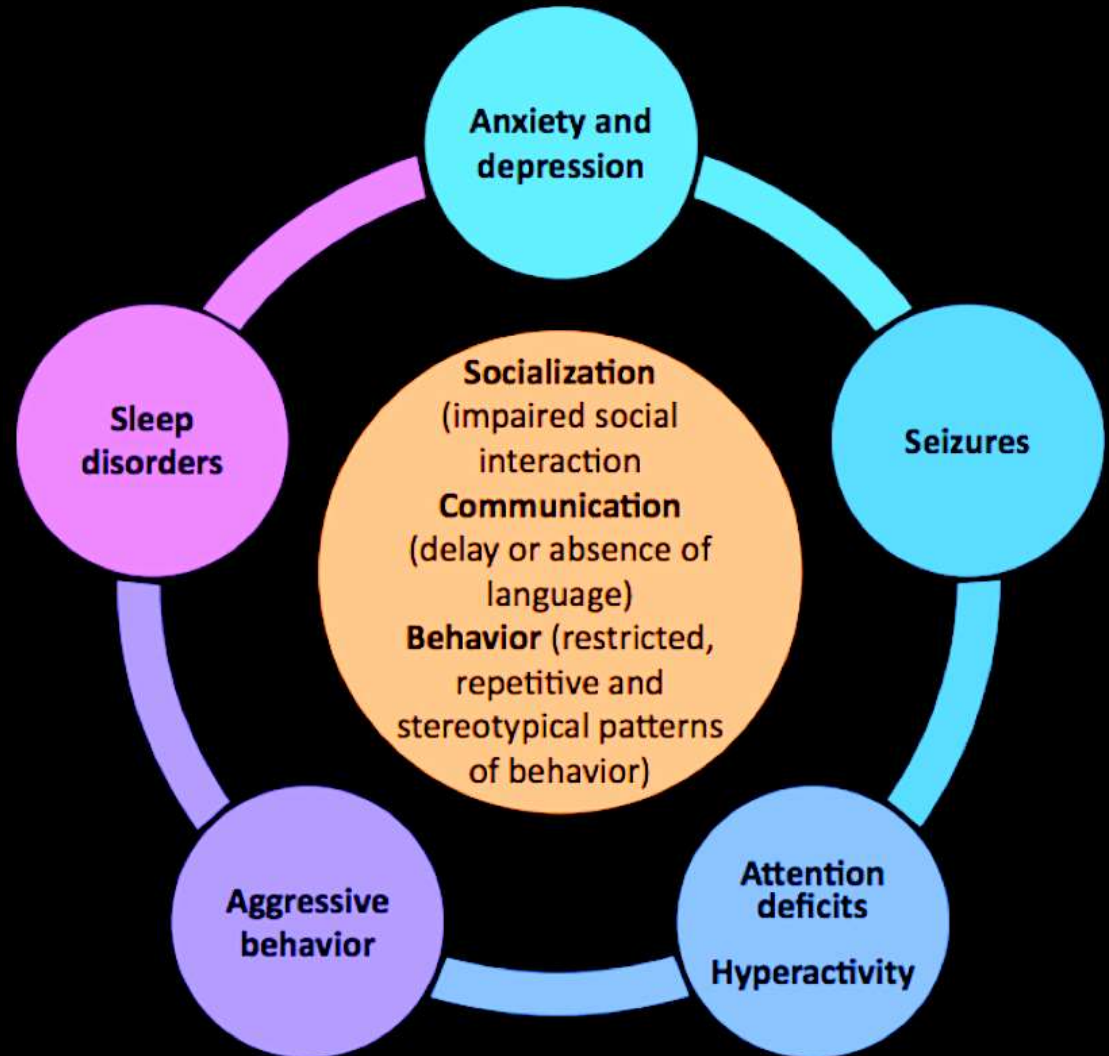
# Autism Spectrum Disorder (ASD)



- Estimated 1 in 44 (CDC 2018 Data)
- 4:1 male/female

# Autism Spectrum Disorder (ASD)

- Core features
  - Social impairment
  - Communication deficits
  - Repetitive behaviors
- Associated features
  - Sensory abnormalities
  - Sleep dysregulation
  - Epilepsy



# An immune subtype of ASD?

- Heightened inflammatory markers

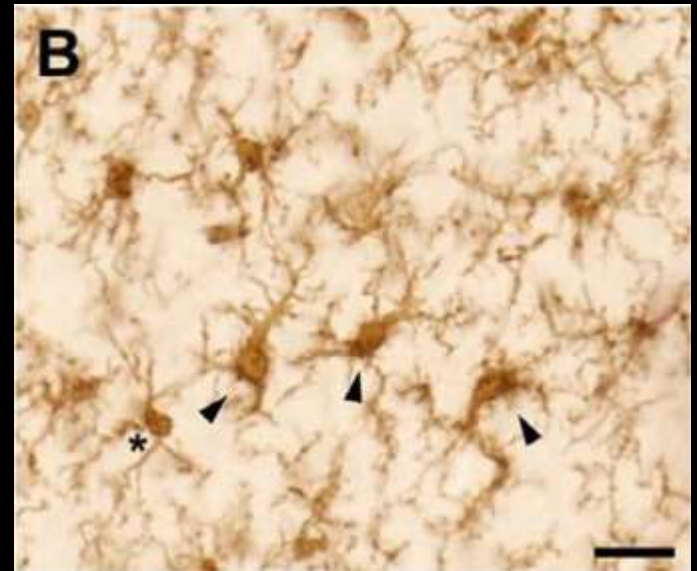
**Table 1.** Characteristics and effect size estimates for cytokine concentrations in autism

Cytokine	Studies (n)	Participants (ASD)	Participants (HC)	% Male (ASD/HC)	Hedges' g	P-value
IL-1 $\alpha$	3	152	93	84/87	-0.128	0.49
IL-1 $\beta$	7	253	241	88/85	0.652	< 0.001
IL-4	3	74	74	96/96	0.137	0.64
IL-6	9	452	383	82/81	0.381	0.03
IL-8	3	150	140	91/89	0.455	0.04
IL-10	3	77	77	92/92	0.026	0.87
IL-12p40	3	150	140	91/89	0.251	0.10
IL-17	3	103	103	78/81	0.193	0.67
IL-23	3	119	99	76/74	-0.262	0.55
IL-1RA	3	66	66	100/100	0.558	0.05
IFN- $\gamma$	6	171	164	77/77	1.044	0.02
TGF- $\beta$ 1	3	144	107	79/70	-1.061	< 0.001
TNF- $\alpha$	6	212	210	92/91	0.227	0.27
G-CSF	3	152	93	84/87	-0.031	0.93
Eotaxin	3	133	111	90/83	0.317	0.01
MCP-1	3	133	111	90/83	0.257	0.05
RANTES	3	133	111	90/83	-0.008	0.97
MIP-1 $\alpha$	3	152	93	84/87	-0.153	0.31
MIP-1 $\beta$	3	152	93	84/87	0.082	0.82

*Masi et al. 2015 Mol. Psychiatry*

# An immune subtype of ASD?

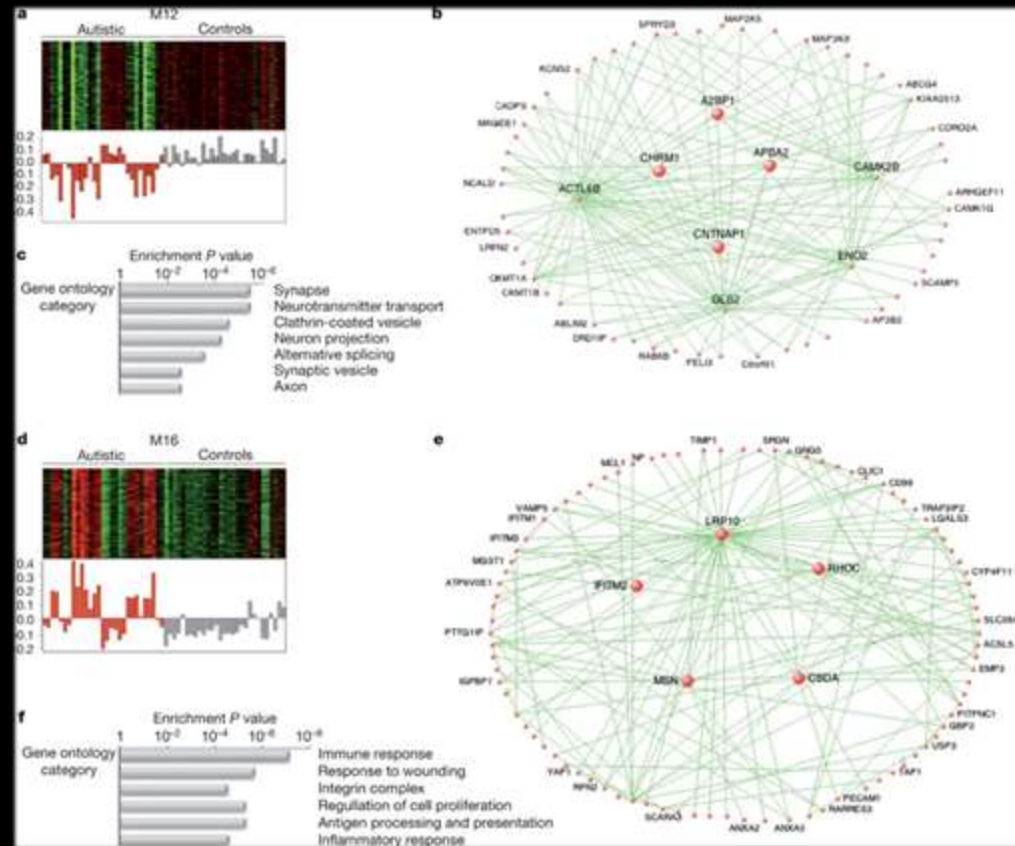
- Heightened inflammatory markers
- Microglia activation



*Lee et al. 2017 Brain, Behavior, and Immunity*

# An immune subtype of ASD?

- Heightened inflammatory markers
- Microglia activation
- Genetic studies
  - Neuron communication
  - Immune processes

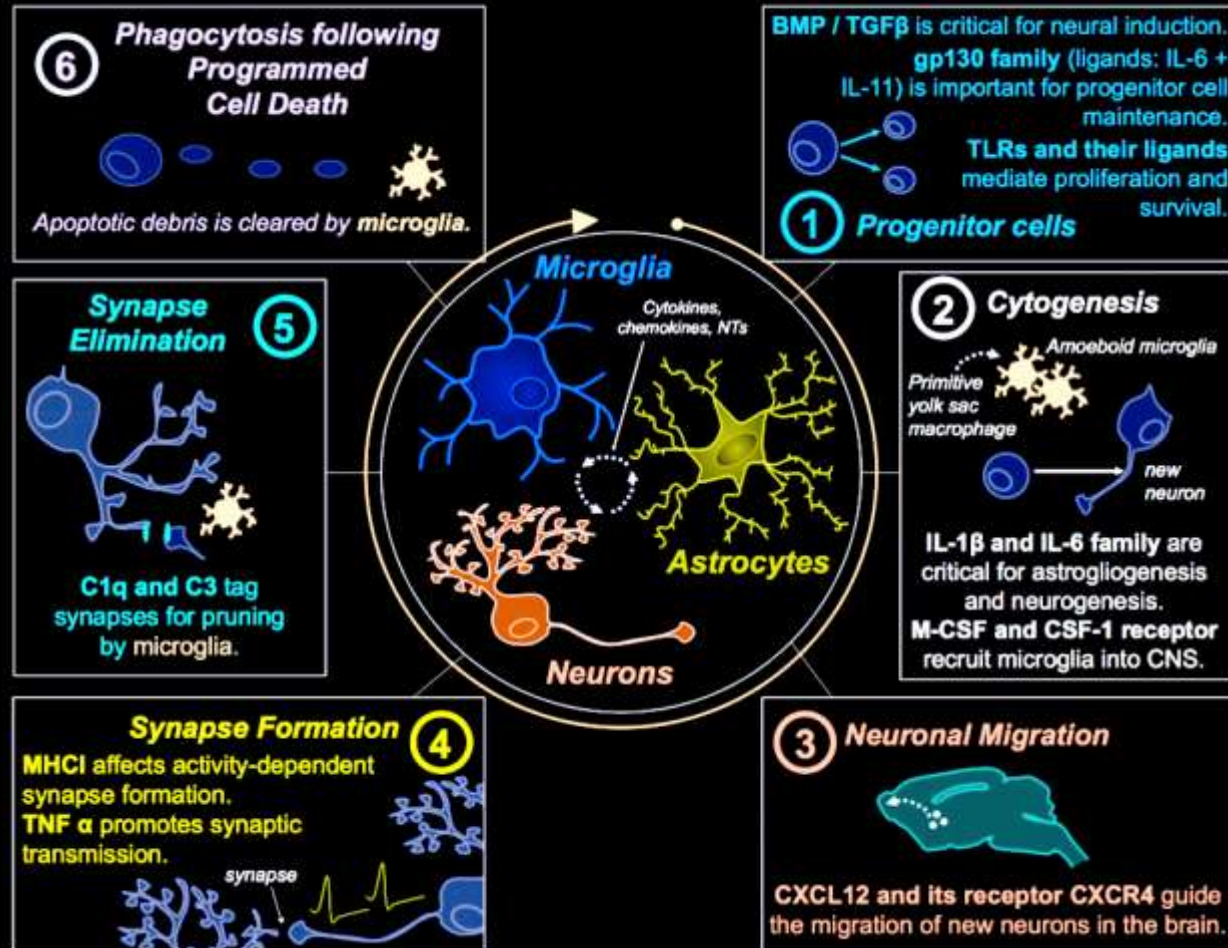


*Voineagu I et al. 2011 Nature*

Question: Why might inflammation contribute to ASD?



# Immune Signaling in the Nervous System



Bilbo & Schwarz 2011

Immune molecules are important in development of nervous system

# Maternal Inflammation and ASD

- Epidemiological associations

Autism Risk Factors	
Maternal Inflammation	Odds Ratio
Bacterial Inf. (2 <sup>nd</sup> trimester)	2.98
Viral Inf. (1 <sup>st</sup> trimester)	1.42
Autoimmune disorder	1.34
SLE (lupus)	2.19
Preeclampsia	2.36

*Atladottir et al 2010*

*Chen et al. 2016*

*Vinet et al. 2015*

*Walker et al. 2015*

# Maternal Inflammation and ASD

- Epidemiological associations
- *Caveat:* Maternal inflammation associated with numerous psychiatric diseases (ADHD, ASD, BPAD, SCZ)

# Maternal Inflammation and ASD

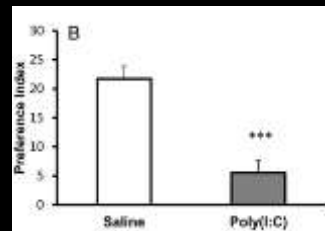
- Epidemiological associations
- *Caveat*: Maternal inflammation associated with numerous psychiatric diseases (ADHD, ASD, BPAD, SCZ)
- Maternal Immune Activation

## Maternal Immune Activation Model

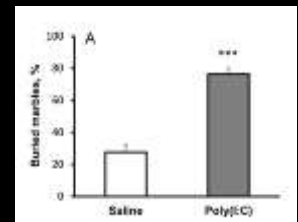
Poly IC  
TLR3 agonist  
viral mimetic

E12.5

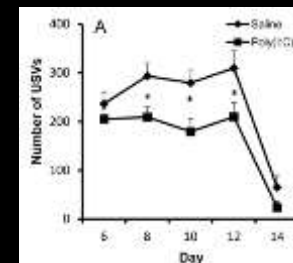
Social Behaviors



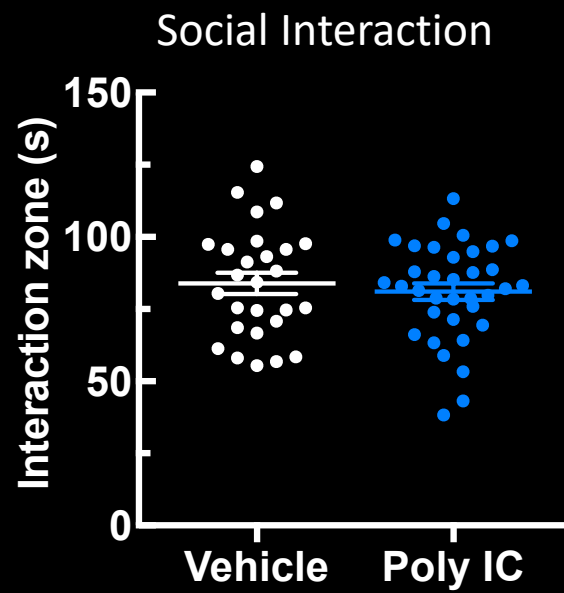
Repetitive Behaviors



Communication



Paul Patterson, PhD



# Our Two-Hit Model

	E12	PND9
VV	Veh	Veh
PV	PolyIC	Veh
VL	Veh	LPS
PL	PolyIC	LPS



1<sup>st</sup> insult to the immune  
system *in utero*

20mg/kg of **Poly IC**  
on day 12.5 of pregnancy



2<sup>nd</sup> insult to the immune  
system *early postnatal*

10mg/kg of **LPS** on postnatal day 9



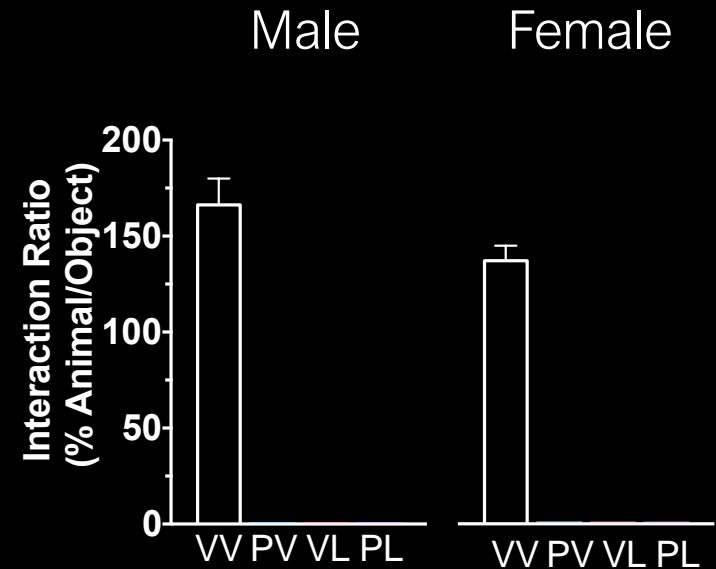
Battery of  
behavioral  
assays

# Perinatal Two-Hit immune model

	E12	PND9
VV	Veh	Veh
PV	PolyIC	Veh
VL	Veh	LPS
PL	PolyIC	LPS

## Social Behavior

### Social Interaction Test – 8 weeks



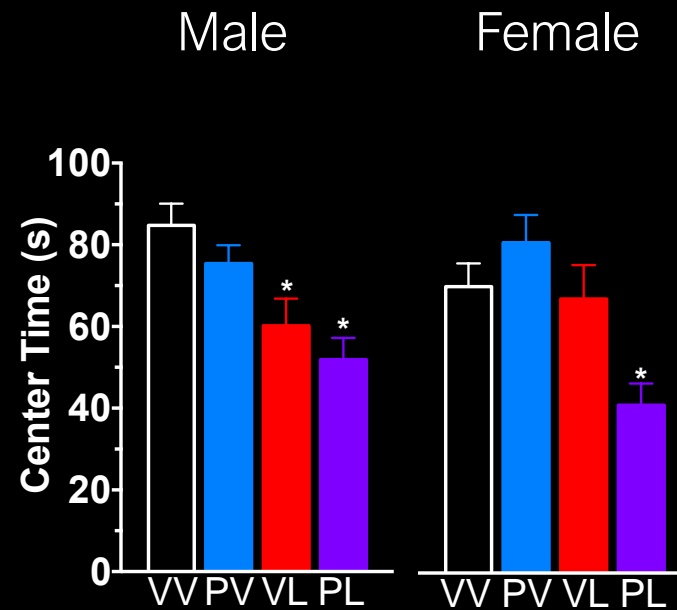
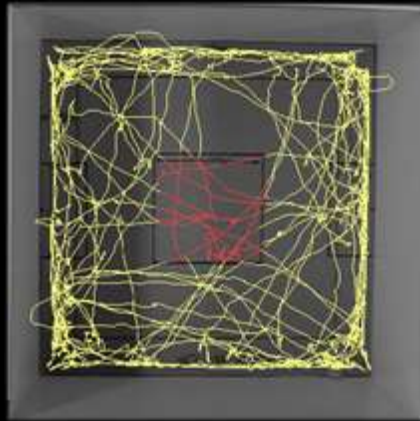
Decreased social interaction in LPS treated males

# Perinatal Two-Hit immune model

	E12	PND9
VV	Veh	Veh
PV	PolyIC	Veh
VL	Veh	LPS
PL	PolyIC	LPS

## Anxiety-like Behavior

Open Field – 6 weeks



Enhanced anxiety-like behavior

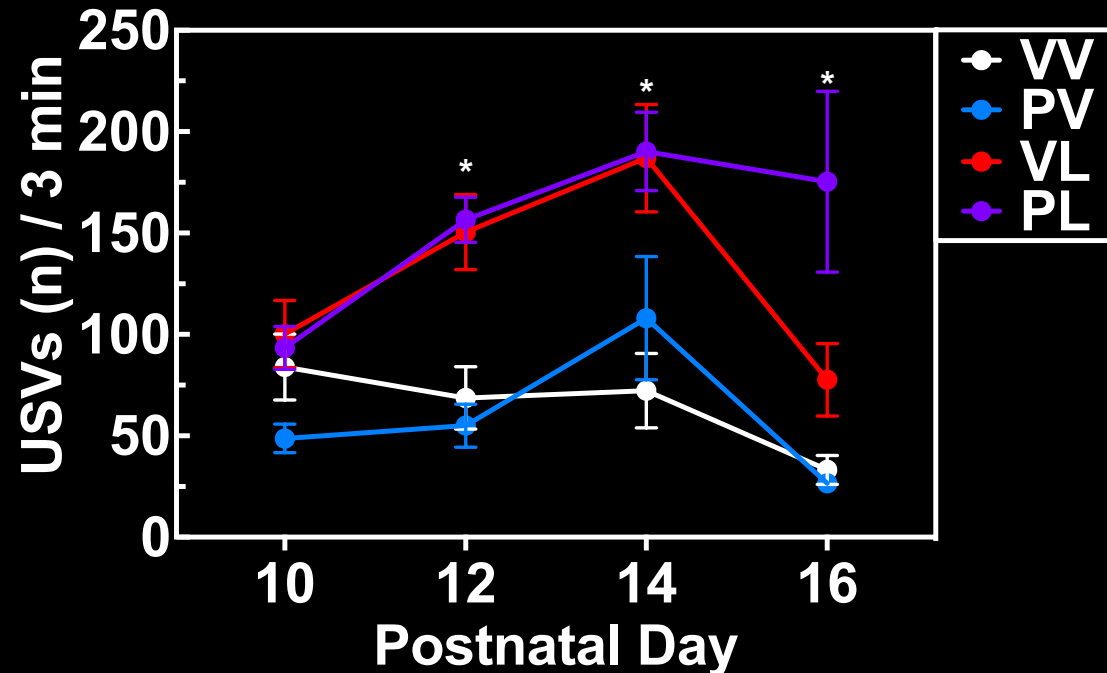
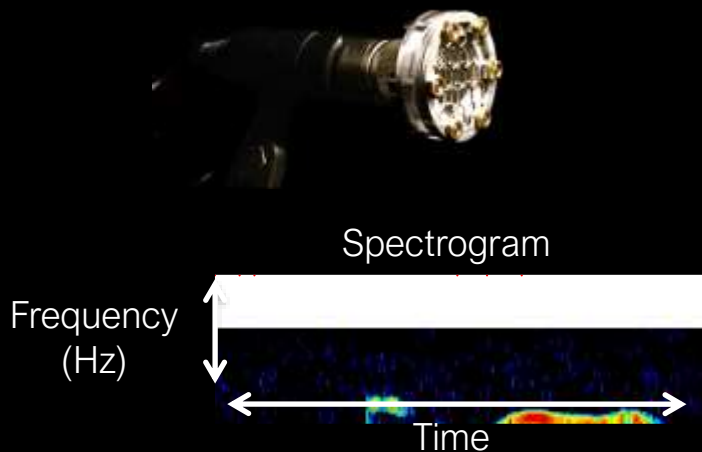


# Perinatal Two-Hit immune model

	E12	PND9
VV	Veh	Veh
PV	PolyIC	Veh
VL	Veh	LPS
PL	PolyIC	LPS

## Communication Behavior

### Pup Ultrasonic Vocalizations— PND 10,12,14,16



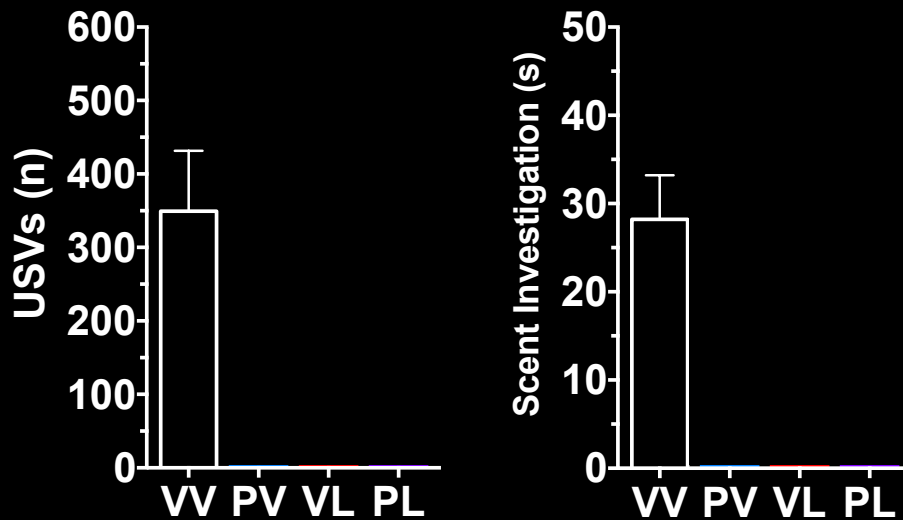
LPS increases USVs in pups

# Perinatal Two-Hit immune model

	E12	PND9
VV	Veh	Veh
PV	PolyIC	Veh
VL	Veh	LPS
PL	PolyIC	LPS

## Communication Behavior

### Social Scent– Week 7



LPS decrease USVs in adults

# Perinatal Two-Hit immune model

	E12	PND9
VV	Veh	Veh
PV	PolyIC	Veh
VL	Veh	LPS
PL	PolyIC	LPS

## Summary

<u>Behavior</u>	Male	Female
Pup USVs	↑ Increase	↑ Increase
Adult USVs	↓ Decrease	<i>No data</i>
Social	↓ Decrease	— No change
Anxiety-like	↑ Increase	↑ Increase

Postnatal LPS results in profound behavioral alterations

Goal: find translational measures for ASD in mice

# EEG/EMG Telemetry

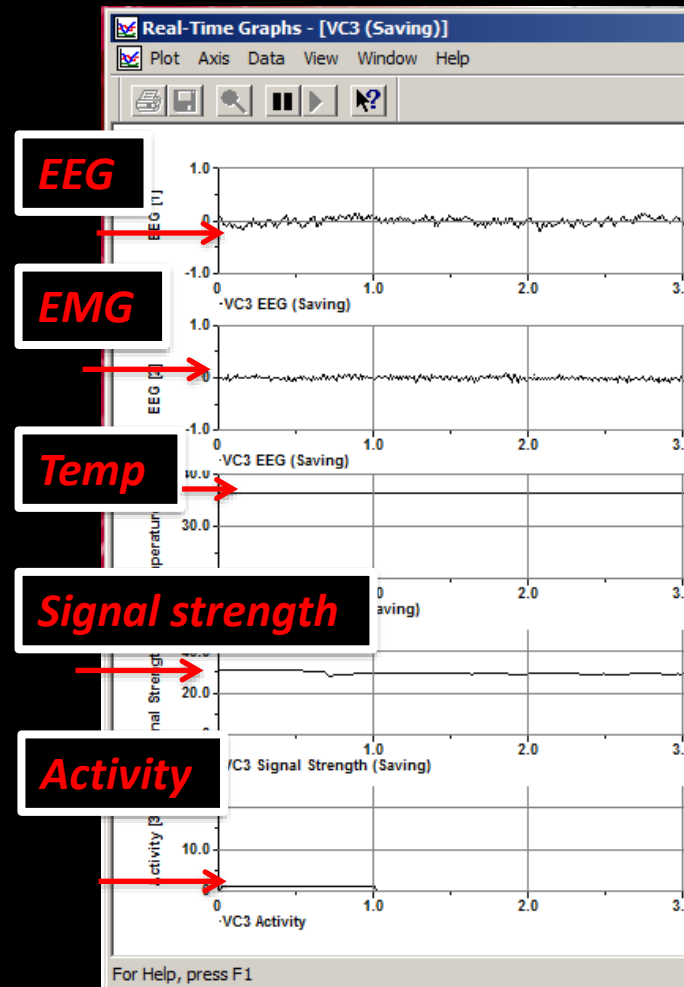
## 24/7 recording for 5 weeks:

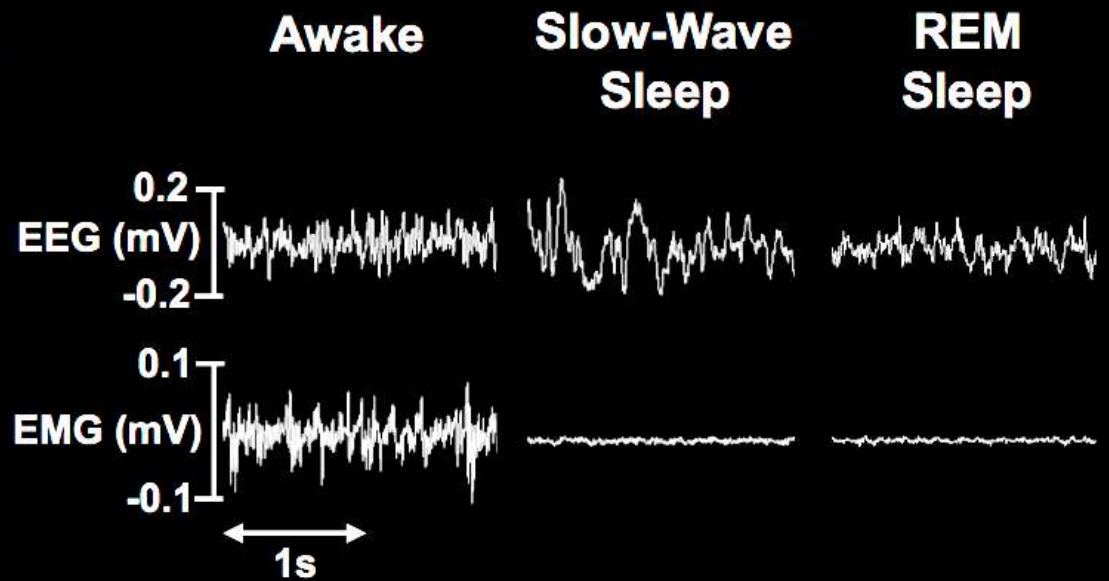
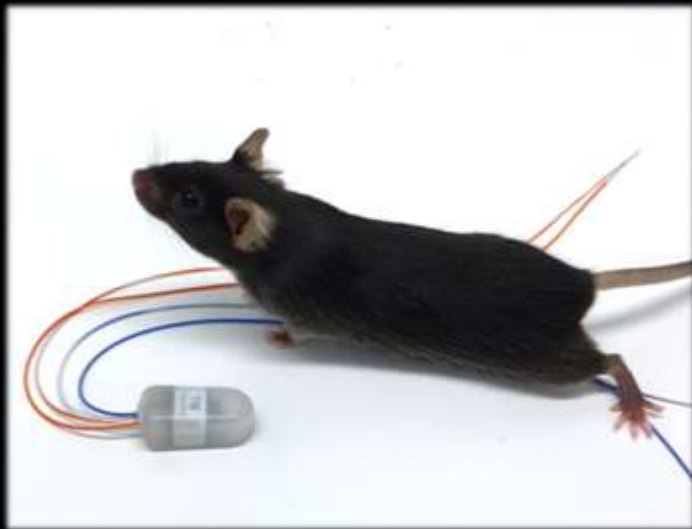
Electroencephalography – frontal / parietal

Electromyography – trapezius

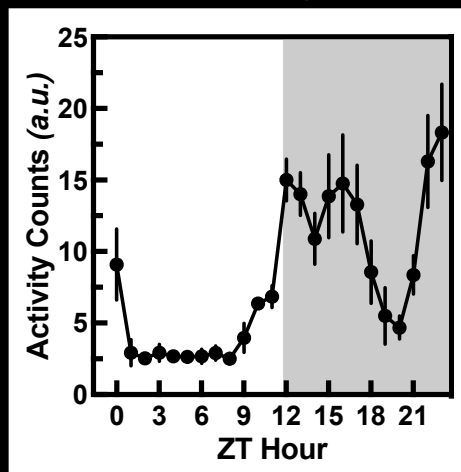
Temperature

Activity

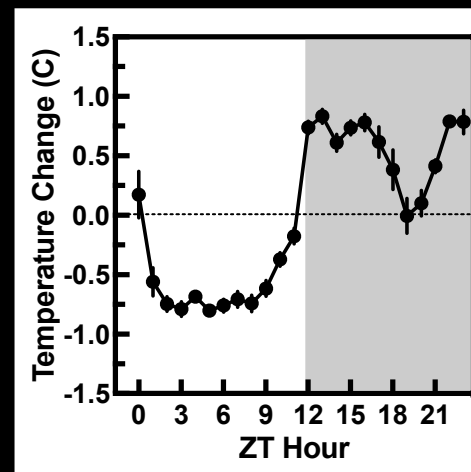




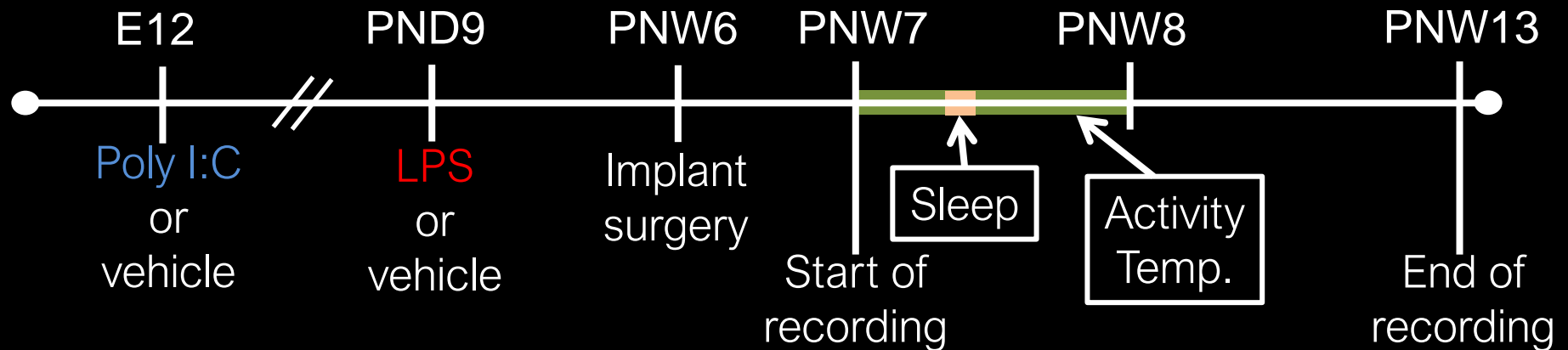
**Activity**



**Temperature**



# Experiment Design



## Sleep Time Points:

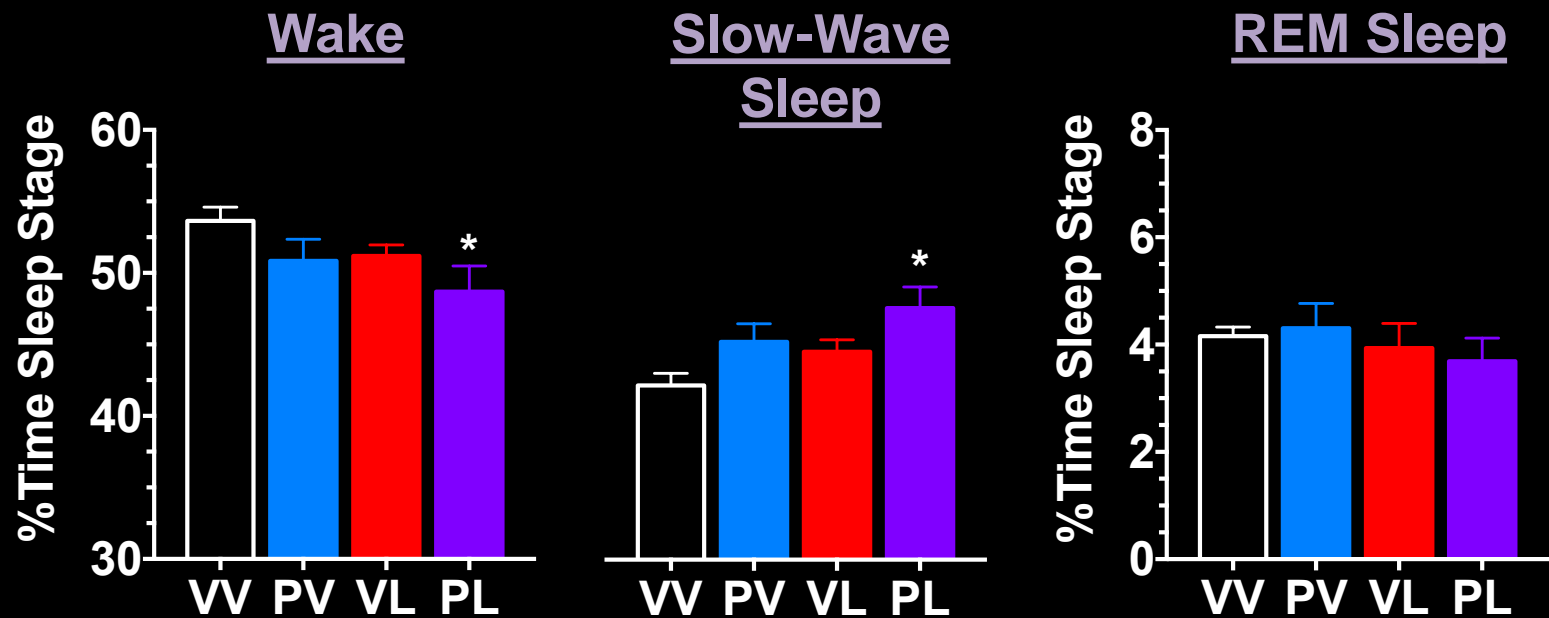
Week 7

Week 13

	E12	PND9
VV	Veh	Veh
PV	PolyIC	Veh
VL	Veh	LPS
PL	PolyIC	LPS

# EEG/EMG Telemetry

## Sleep Architecture



LPS + Poly IC increases slow-wave sleep



+0.5mV  
**EEG**  
-0.5mV

Spike-Wave Discharge

# Epileptiform Activity

*Example 1:*

**EEG**



## **Spike-Wave Discharges (SWDs)**

Spike: 3X background,  $>100 \mu\text{V}$

Spike-Wave Discharge:  $\geq 4$  spikes

Avg. Duration:  $1.3 \pm 0.7\text{s}$

Peak Frequency: 6-7 Hz

*Example 2:*

**EEG**



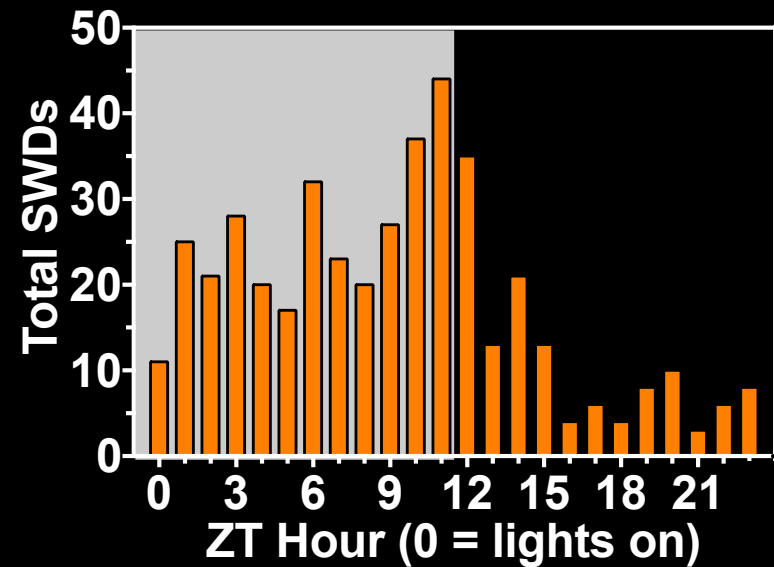
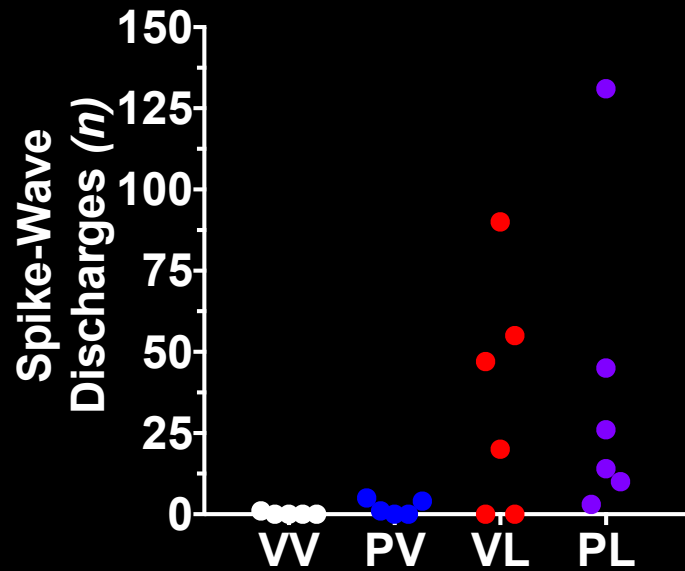
**EMG**



# Epilepsy and ASD

- Epilepsy in ASD estimates: 5-46%
- Epileptiform activity in ASD in absences of seizures
  - One study found in 900 children with no known epilepsy 61% had epileptiform EEG during sleep (*Chez et al. 2006*)
- In ASD, epileptiform activity was associated with higher levels of IL-6 (*Jacome et al. 2016*)

# Epileptiform Activity



## Postnatal LPS results in epileptiform activity

# EEG Power Spectrum



## Normal Adult Brain Waves

Awake with  
mental activity



Beta  
14-30 Hz

Awake and  
resting



Alpha  
8-13 Hz

Sleeping



Theta  
4-7 Hz

Deep sleep



Delta  
<3.5 Hz

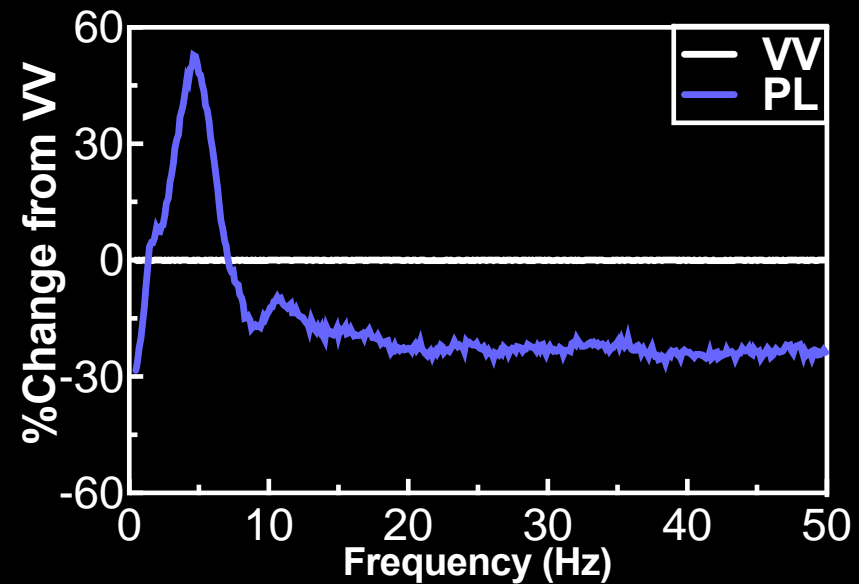
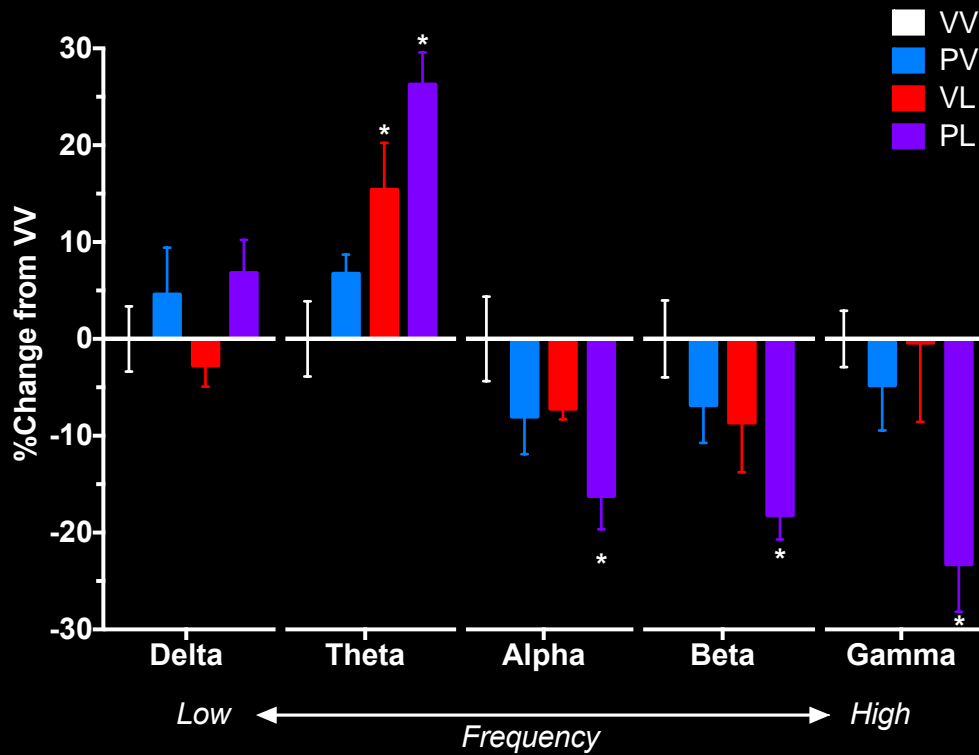


1 sec

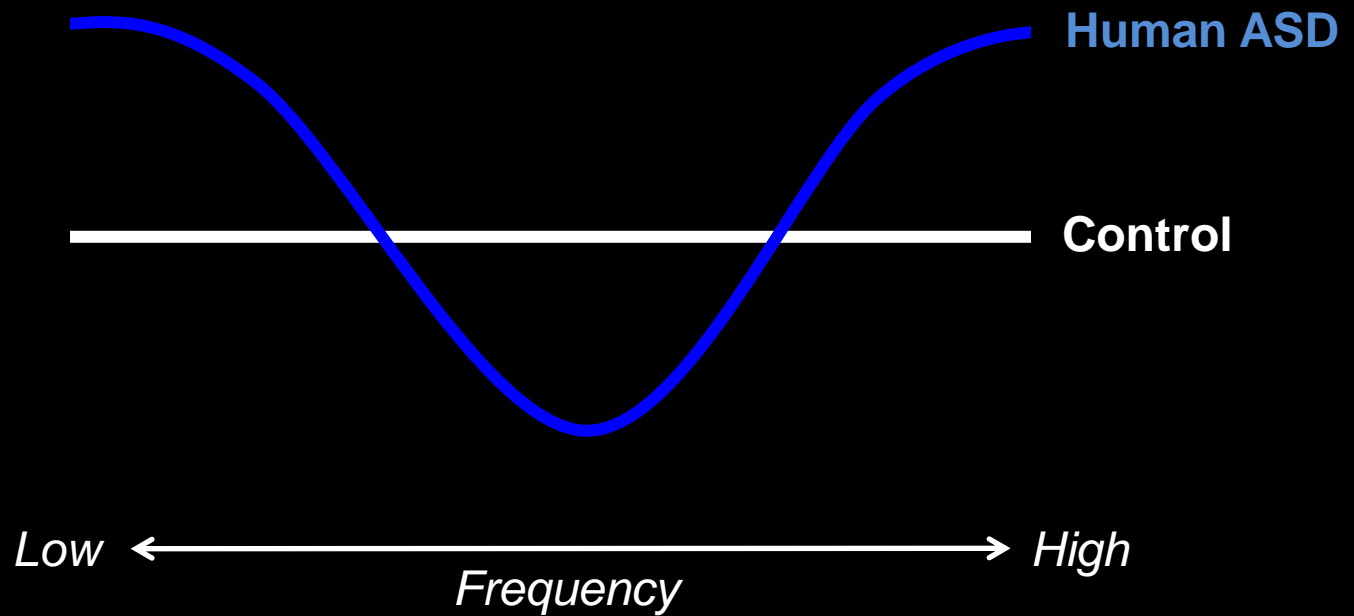
# EEG Power Spectrum

# EEG Power Spectrum

Wake

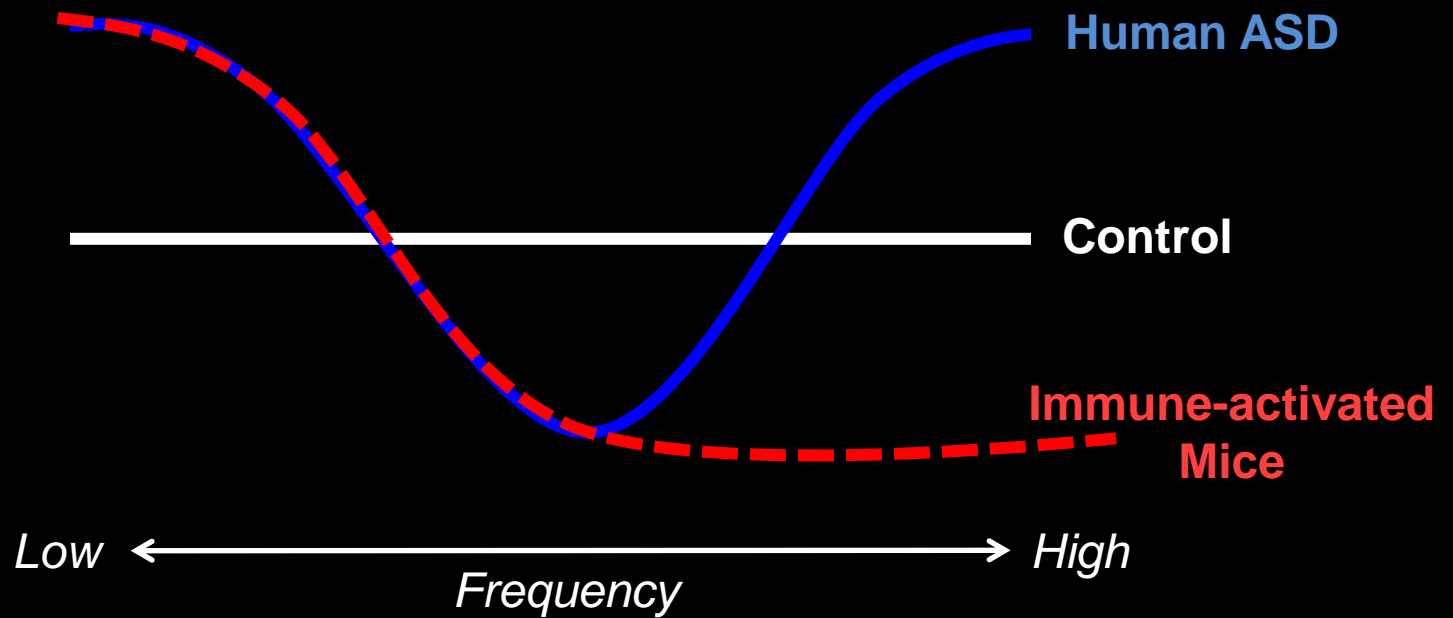


# EEG Power Spectrum





# EEG Power Spectrum



# EEG/EMG Telemetry

## Summary

### Poly IC + LPS Two-Hit Model

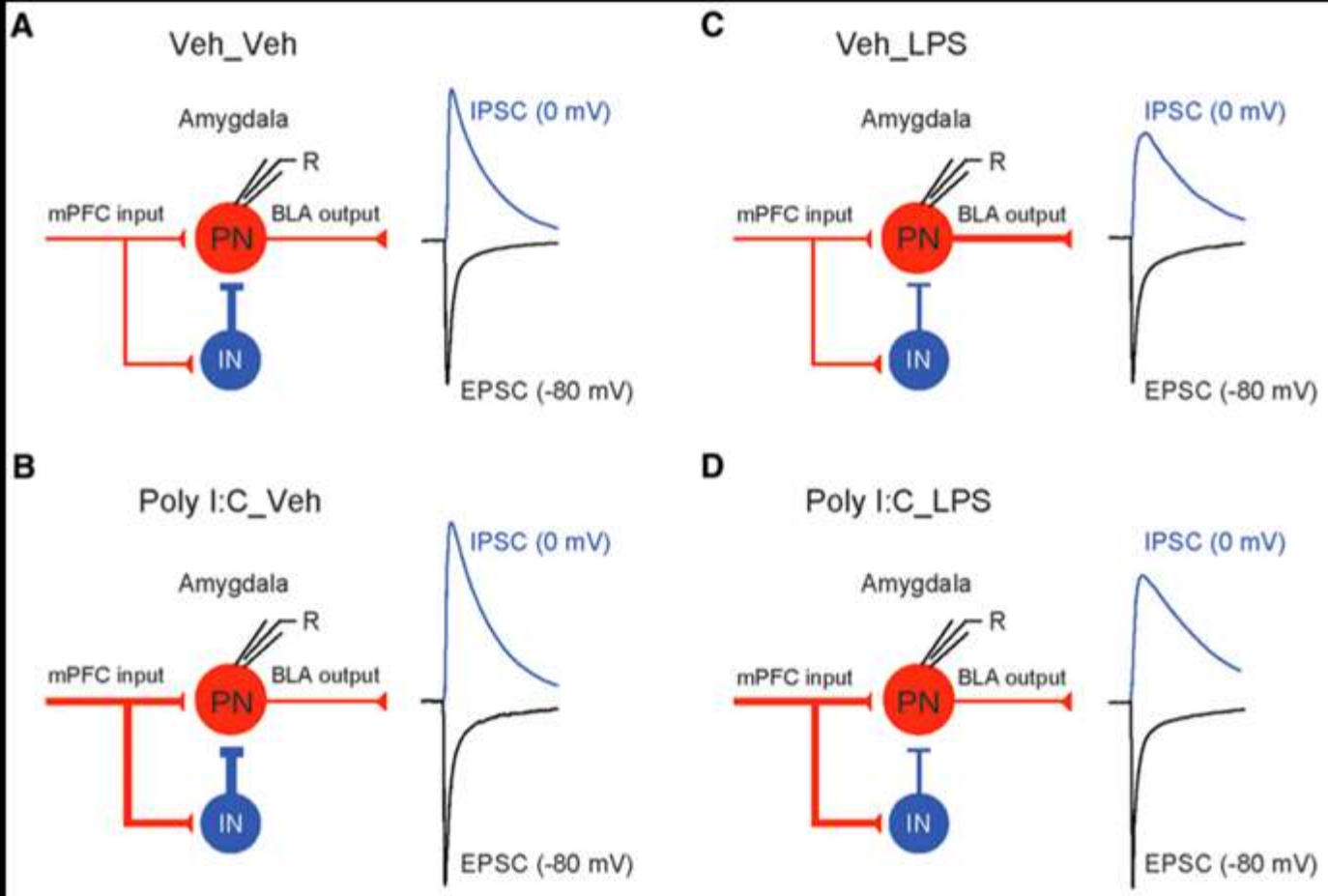
- Increased Slow-wave sleep
- Epileptiform activity (spike-wave discharges)
- EEG spectral power changes
- Persists 5 weeks later

## Maternal and Early Postnatal Immune Activation Produce Dissociable Effects on Neurotransmission in mPFC-Amygdala Circuits.

Li Y<sup>1</sup>, Missig G<sup>1</sup>, Finger BC<sup>1</sup>, Landino SM<sup>1</sup>, Alexander AJ<sup>1</sup>, Mokler EL<sup>1</sup>, Robbins JO<sup>1</sup>, Manasian Y<sup>1</sup>, Kim W<sup>1</sup>, Kim KS<sup>1</sup>, McDougale CJ<sup>2</sup>, Carlezon WA Jr.<sup>3</sup>, Bolshakov VY<sup>3</sup>.

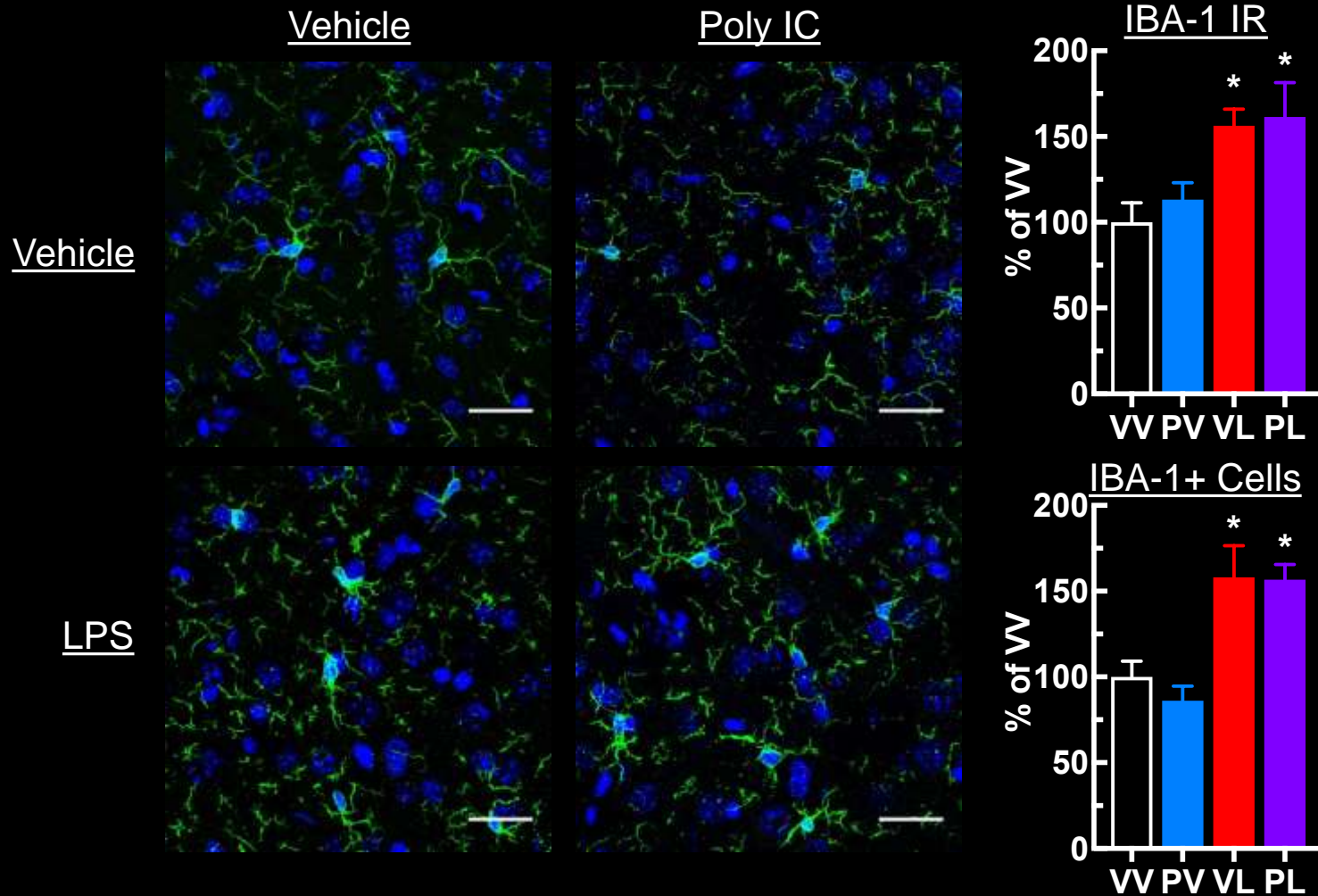


Yan Li, PhD



*Next Step:* Is this phenotype from ongoing or developmental immune dysregulation?

# Perinatal Two-Hit immune model



Increased microglia density

# Perinatal Two-Hit immune model



Woori Kim, PhD

Protein		mPFC			AMY			HP			TM			
Compared to VV		PV	VL	PL	PV	VL	PL	PV	VL	PL	PV	VL	PL	
Pro-inflammatory	IL-6	↔	↔	↔	↔	↔	↑	↔	↔	↔	↔	↑	↑	Male
	IL-1 $\beta$	↑	↑	↑↑	↔	↑	↑↑	↔	↔	↔	↔	↔	↑↑	
	Iba-1	↑	↑	↑	↔	↑	↑	↔	↑	↔	↔	↑	↑	
	GFAP	↑	↑	↑	↔	↑	↑	↔	↑	↑	↔	↑	↑	
	TSPO	↔	↑	↑	↔	↑	↑	↔	↑	↔	↔	↑	↑	
Anti-	IL-10	↓	↓	↓	↔	↓	↓	↔	↓	↓	↔	↔	↓	
	TGF- $\beta$ 1	↔	↓	↓	↔	↓	↓	↔	↓	↓	↓	↓	↓	
Compared to VV		PV	VL	PL	PV	VL	PL	PV	VL	PL	PV	VL	PL	
Pro-inflammatory	IL-6	↔	↔	↔	↑	↑	↑	↔	↔	↔	↔	↔	↔	Female
	IL-1 $\beta$	↑	↑	↔	↑	↑	↔	↔	↔	↔	↔	↑	↔	
	Iba-1	↔	↑	↔	↔	↑	↑	↔	↔	↔	↔	↑	↑	
	GFAP	↔	↔	↑	↔	↔	↔	↔	↑	↑	↔	↔	↔	
	TSPO	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	↔	
Anti-	IL-10	↑	↑	↑	↔	↑	↔	↑	↑	↔	↔	↑	↑	
	TGF- $\beta$ 1	↔	↔	↑	↔	↑	↑	↑	↑	↔	↔	↑	↑	

Single arrow, < 2 fold;

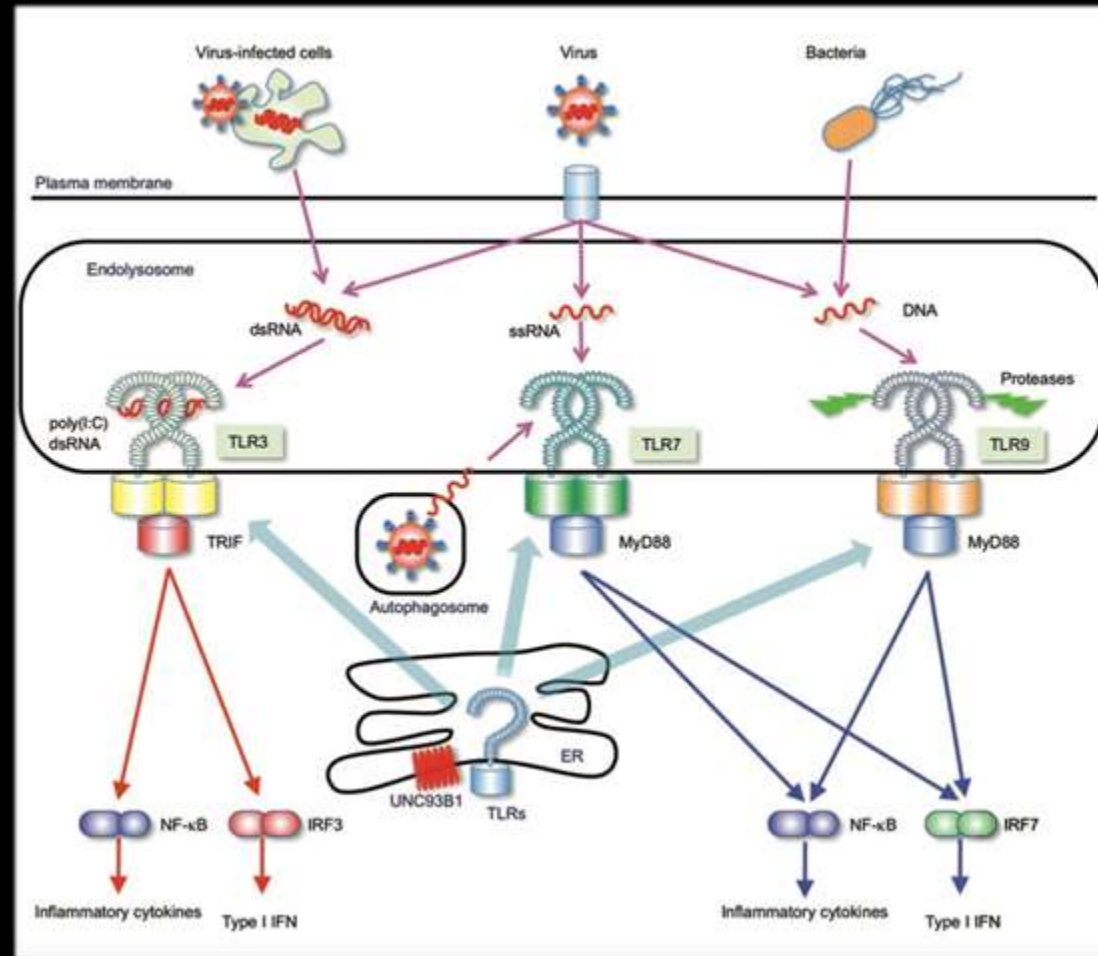
Double arrow, > 2 fold

Increased inflammatory-related proteins in brain

## PART 2:

Goal: examine novel maternal immune insults

# Toll-like receptor

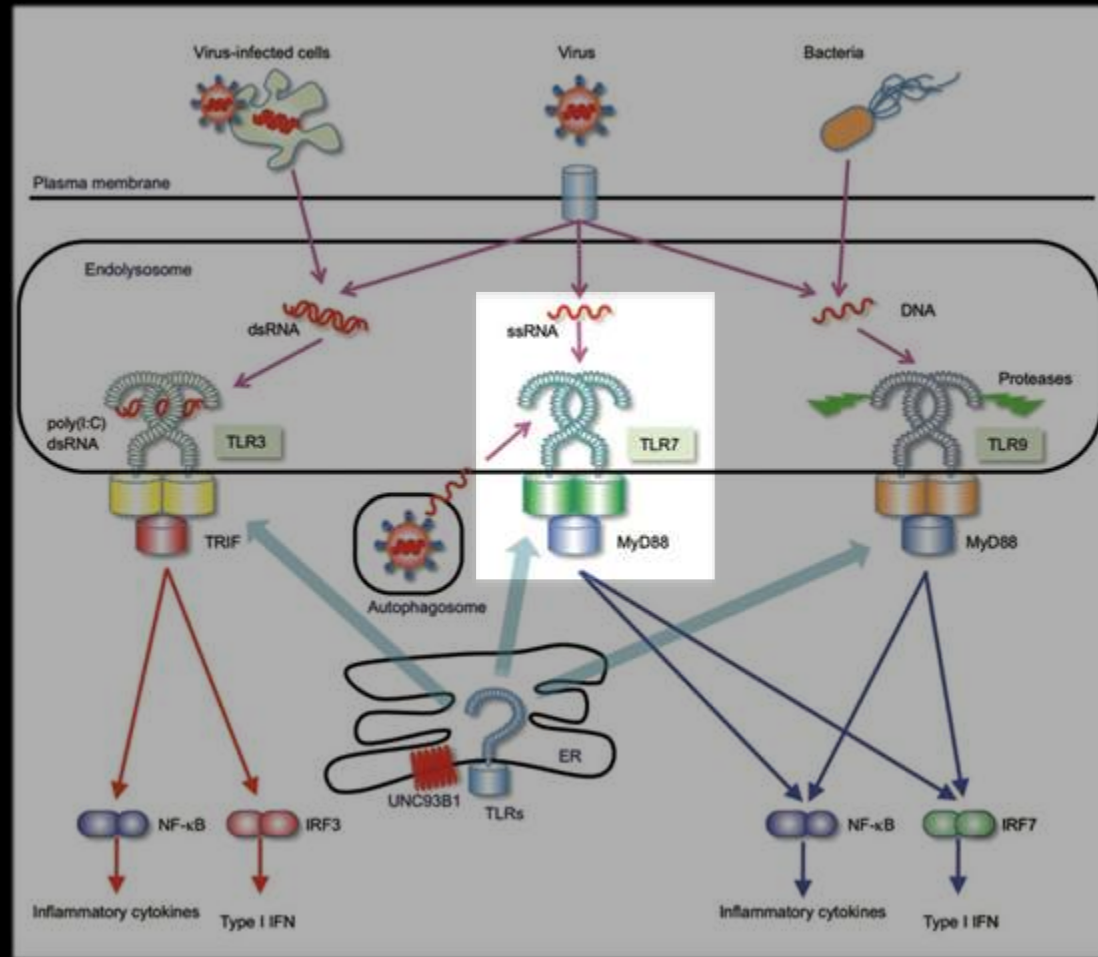


Kawai & Akira 2010



# Toll-like receptor 7

- Toll-like receptor 7 (TLR7)
  - Recognizes ssRNA

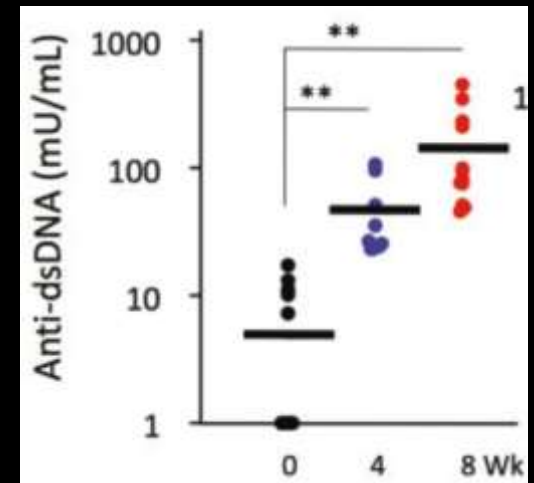
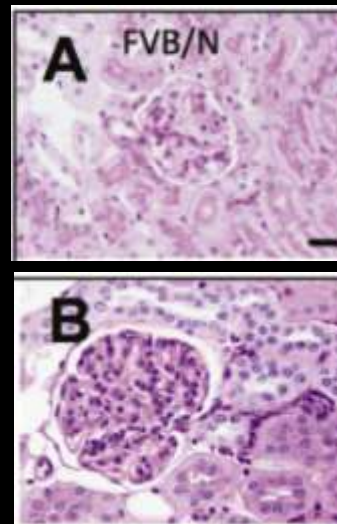


# Toll-like receptor 7

- Toll-like receptor 7 (TLR7)
  - Recognizes ssRNA
- Involvement in autoimmunity

## Autism Risk Factors

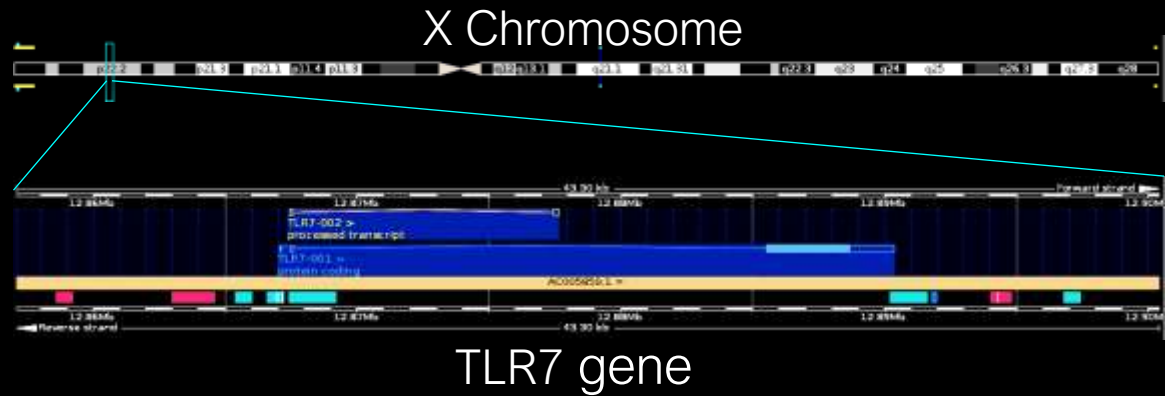
Maternal Inflammation	Odds Ratio
Bacterial Inf. (2 <sup>nd</sup> trimester)	2.98
Viral Inf. (1 <sup>st</sup> trimester)	1.42
Autoimmune disorder	1.34
SLE (lupus)	2.19
Preeclampsia	2.36



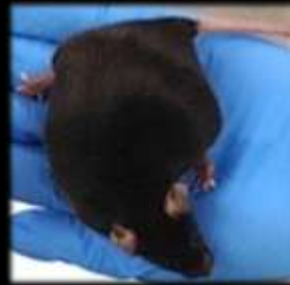
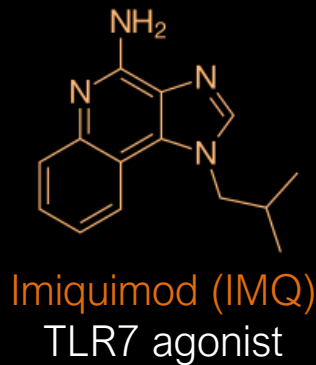
Consequences of repeated TLR7 activation  
Yokogawa et. al 2014

# Toll-like receptor 7

- Toll-like receptor 7 (TLR7)
  - Recognizes ssRNA
- Involvement in autoimmunity
- Encoded by X Chromosome



# Prenatal TLR7 Activation

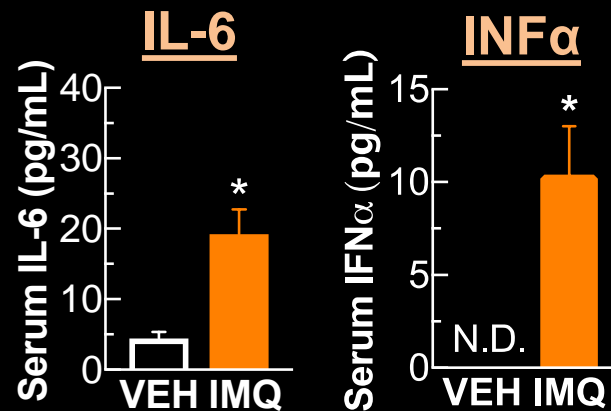


5mg/kg of **Imiquimod**  
on day 12, 14, 16 of pregnancy

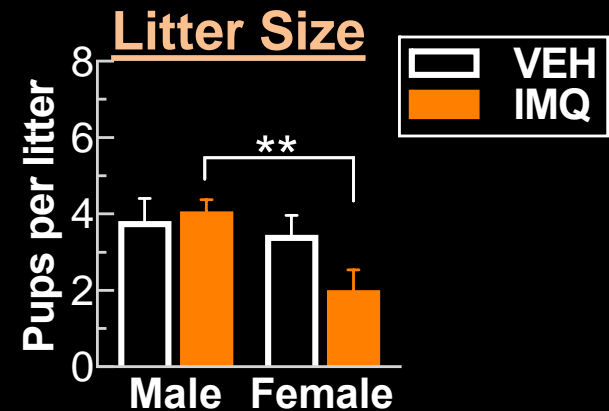
offspring  
→

Battery of  
behavioral  
assays

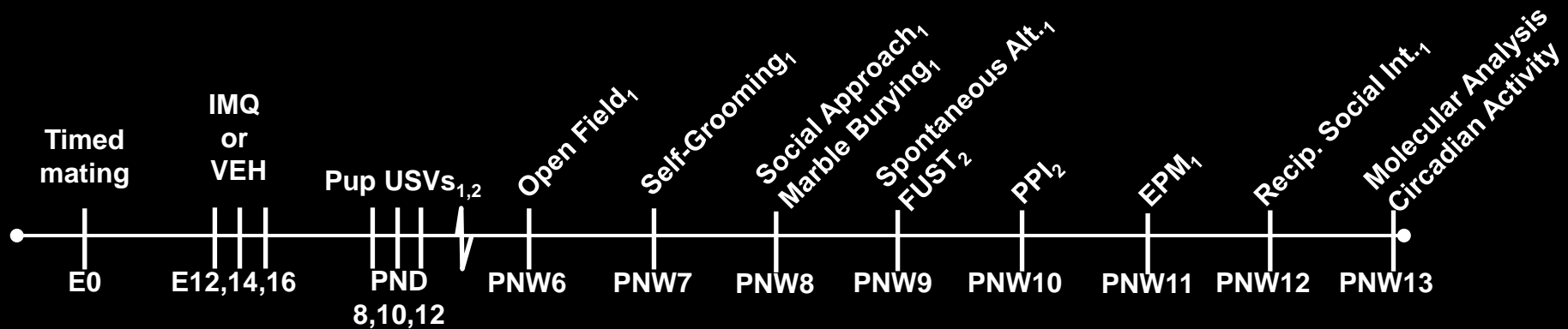
# Prenatal TLR7 Activation



IMQ induces a maternal inflammatory response



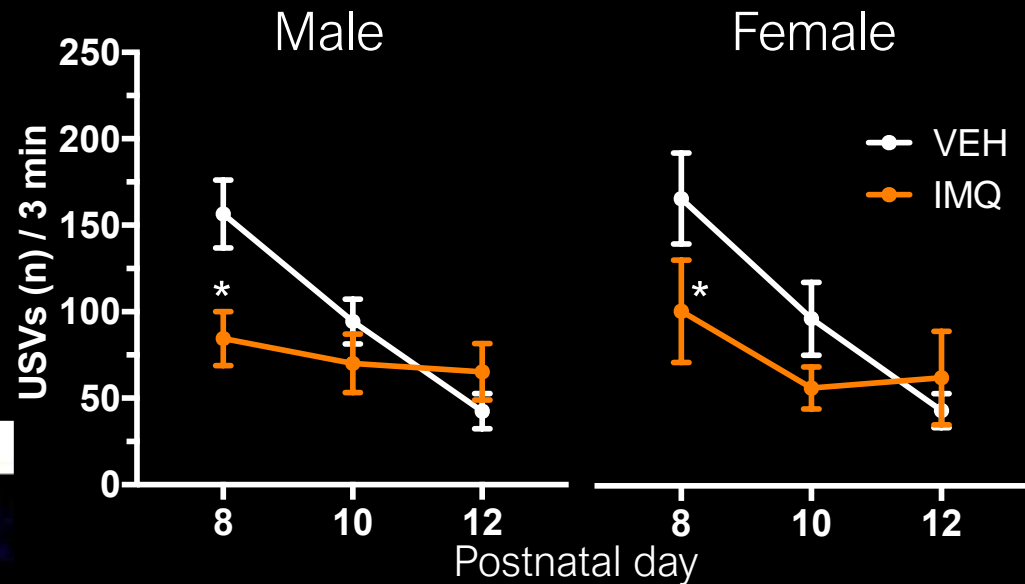
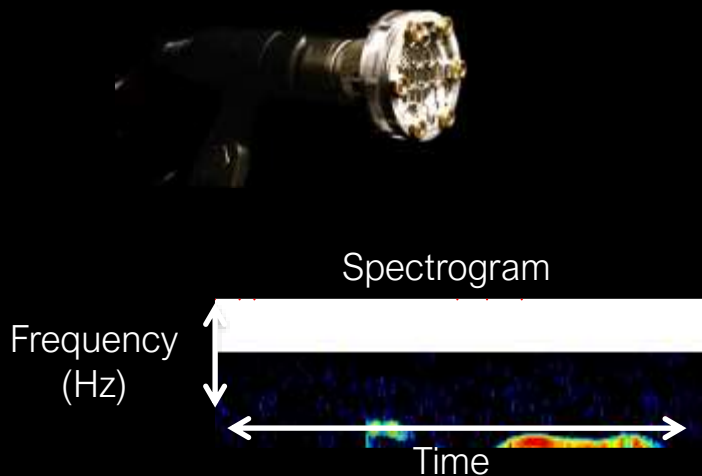
IMQ leads to a selective loss of female embryos



# Prenatal TLR7 Activation

## Communication-related Behavior

### Pup Ultrasonic Vocalizations—PND 8, 10, 12



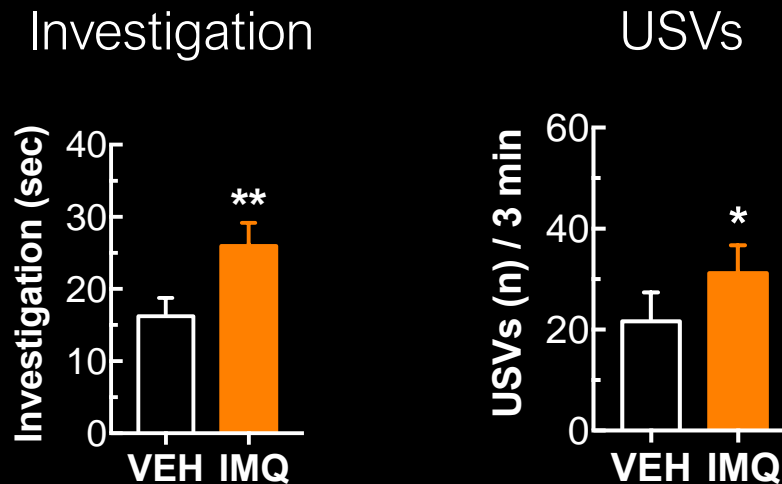
Altered communication

# Prenatal TLR7 Activation

## Communication-related Behavior



**Social Scent**— 9 weeks

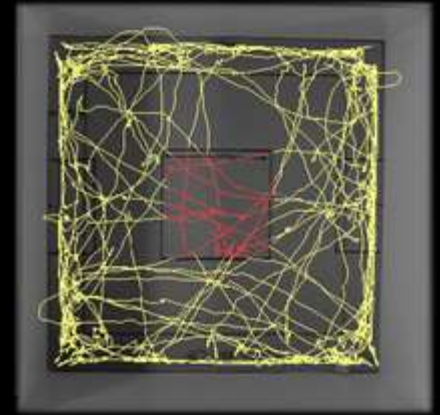


Increased social scent investigation

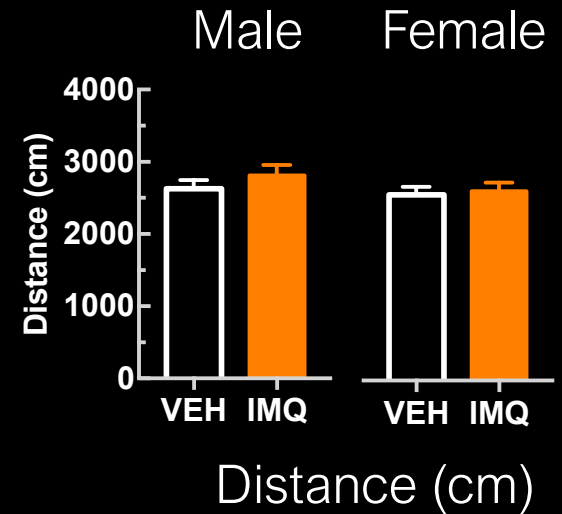
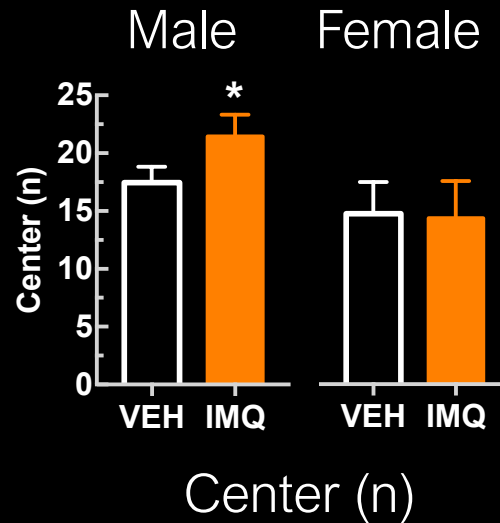
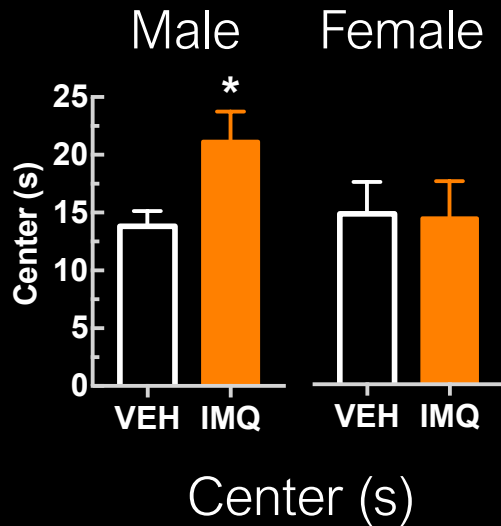


# Prenatal TLR7 Activation

## Anxiety-Like Behavior



### Open Field – 6 weeks

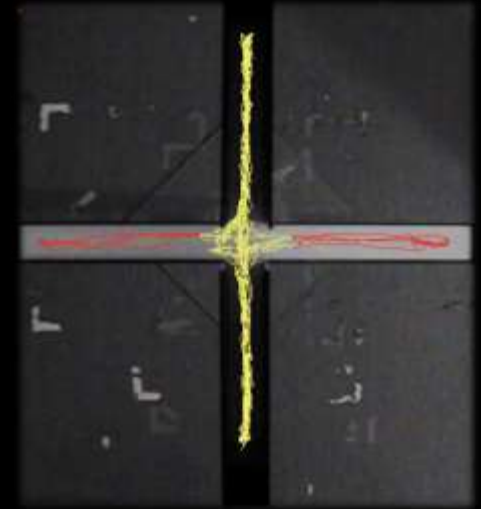
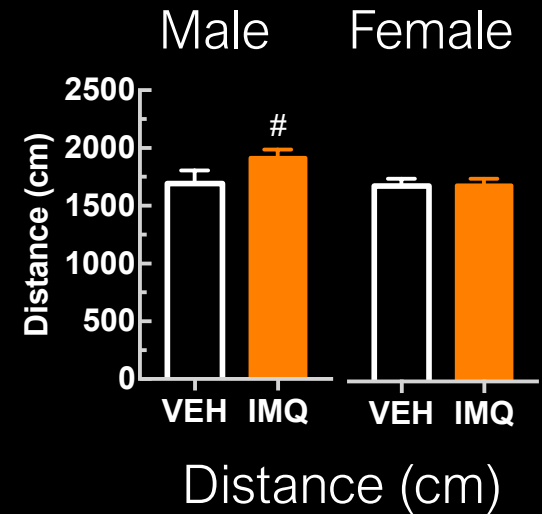
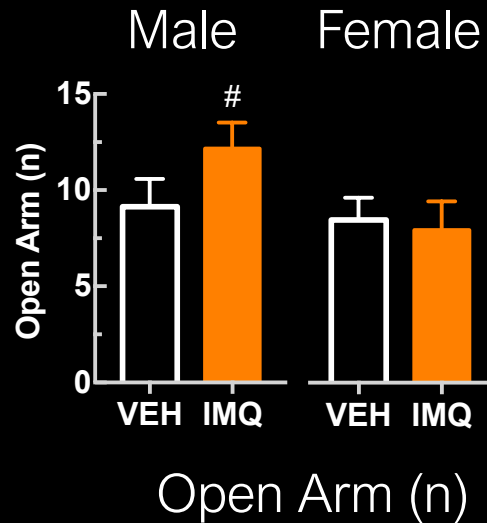
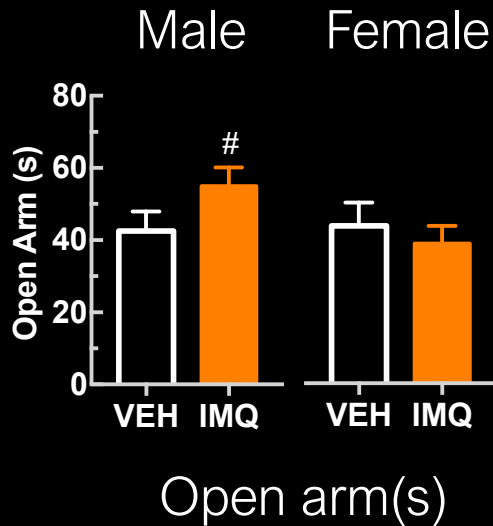


Males show less anxiety-like behavior

# Prenatal TLR7 Activation

## Anxiety-Like Behavior

### Elevated Plus Maze – 11 weeks



Males show less anxiety-like behavior

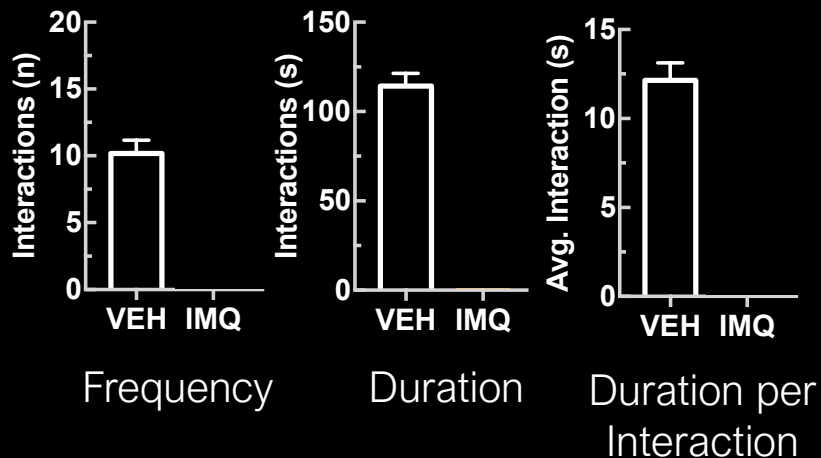
# Prenatal TLR7 Activation

## Social Behavior



## Social Interaction Test – 8 weeks

Male



Fragmented social behavior

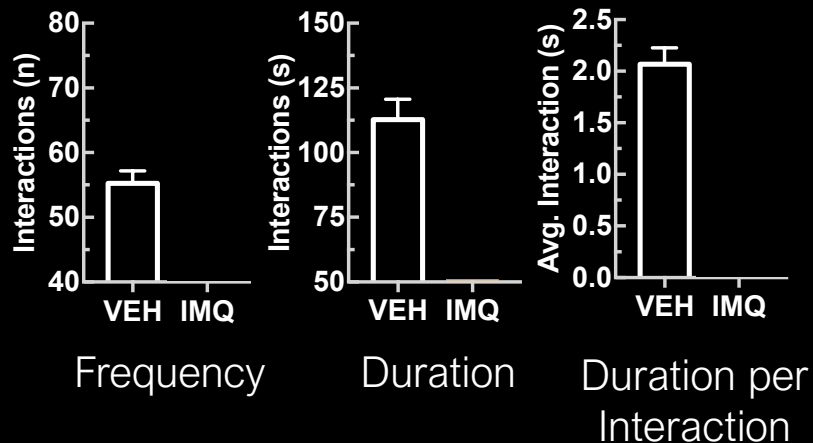
# Prenatal TLR7 Activation

## Social Behavior

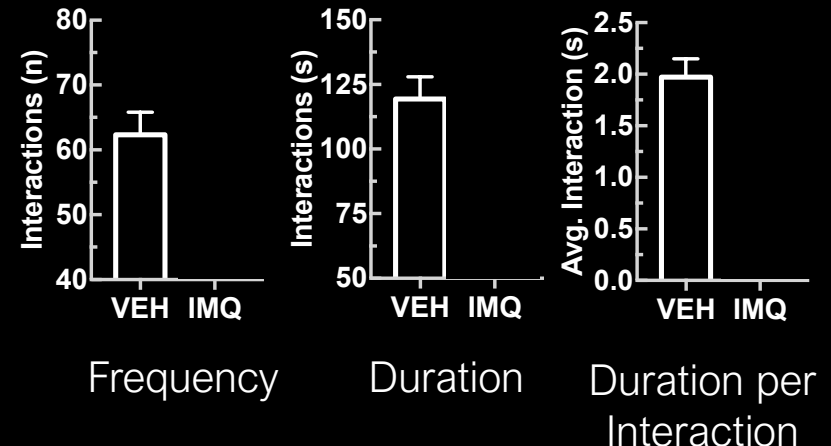


## Social Dyad Test – 12 weeks

### Male



### Female



Fragmented social behavior

# Prenatal TLR7 Activation

## Summary

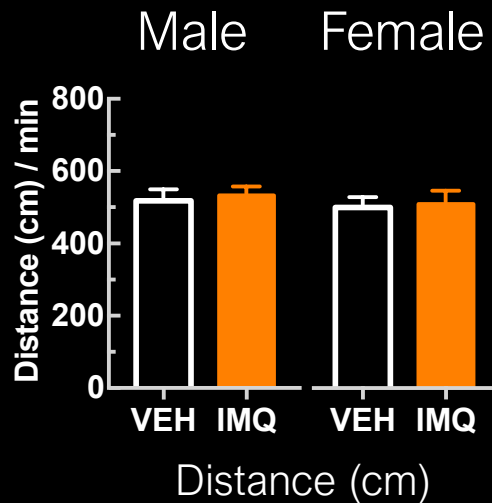
<u>Behavior</u>	Male	Female
Pup USVs	↓ Decrease	↓ Decrease
Anxiety-like	↓ Decrease	— No change
Repetitive	↑ Increase	↑ Increase
Social Interaction	↑↓ Fragmented	↑↓ Fragmented
Social Scent	↑ Increased	

Greater behavioral disturbances in males

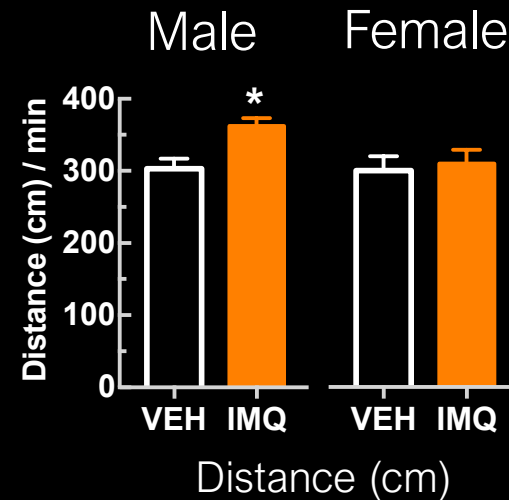
# Prenatal TLR7 Activation

## Activity

### Open Field

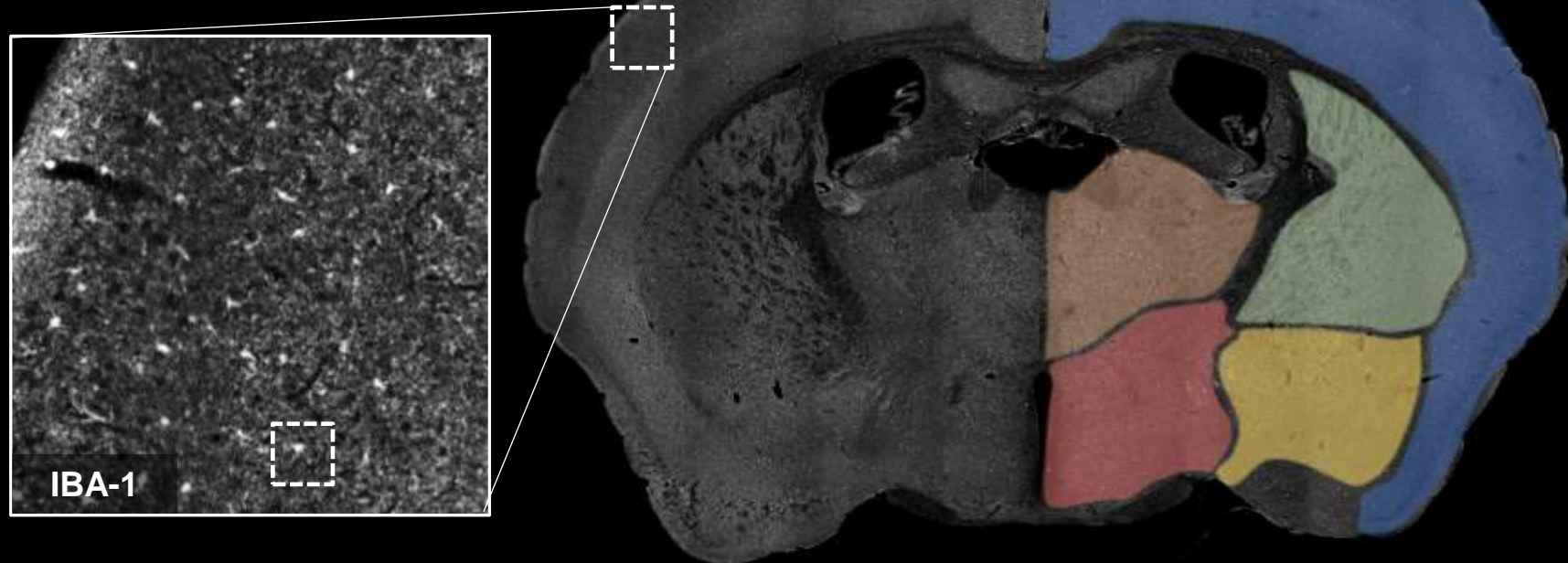


### Social Interaction Test

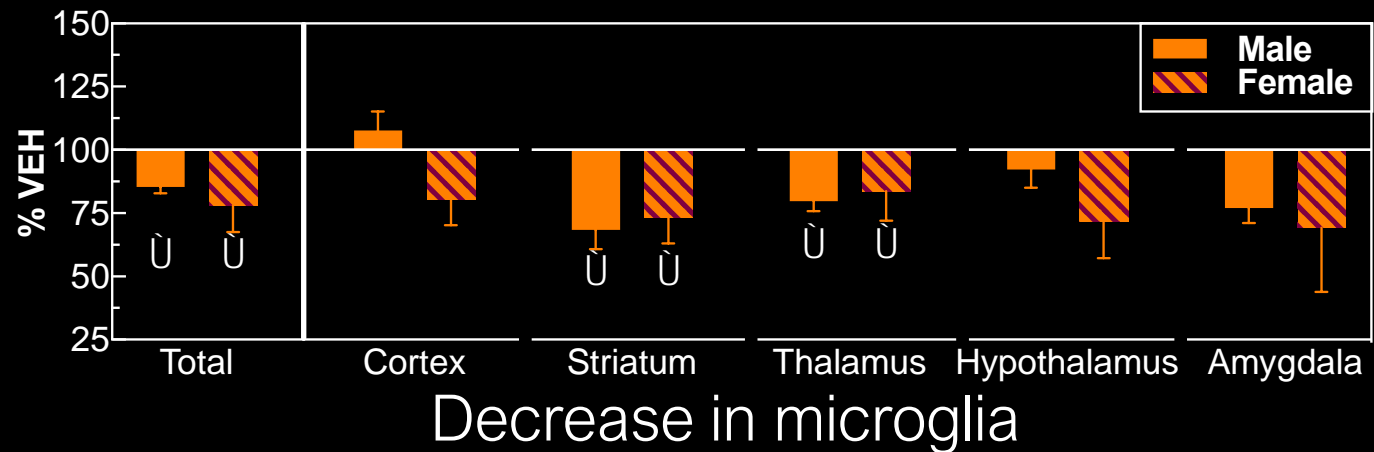


Conditional hyperactivity in males

# Prenatal TLR7 Activation

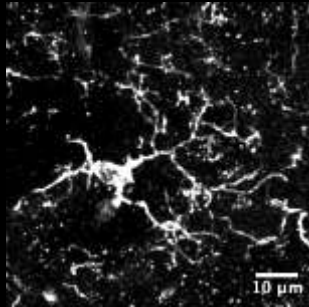


## IBA-1 Quantification



# Prenatal TLR7 Activation

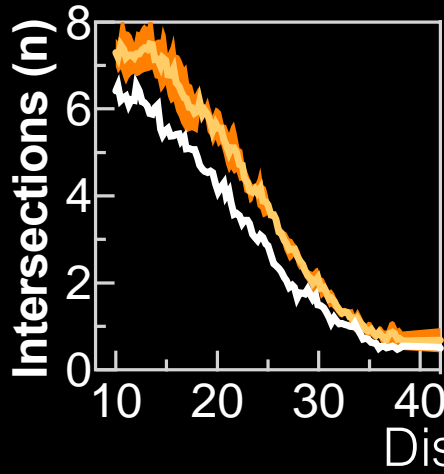
Microglia



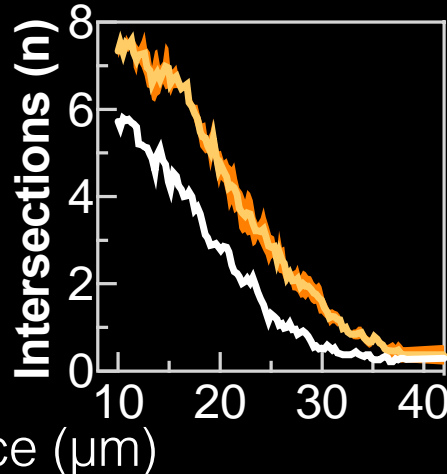
Sholl analysis



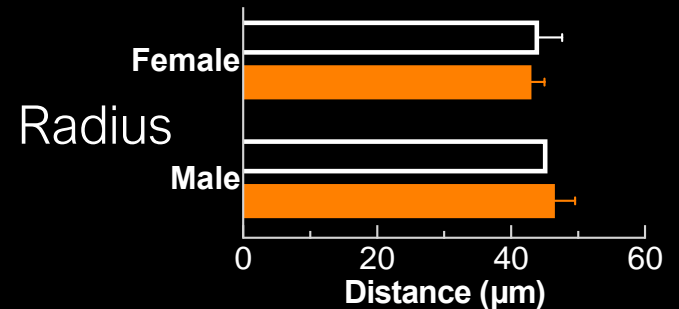
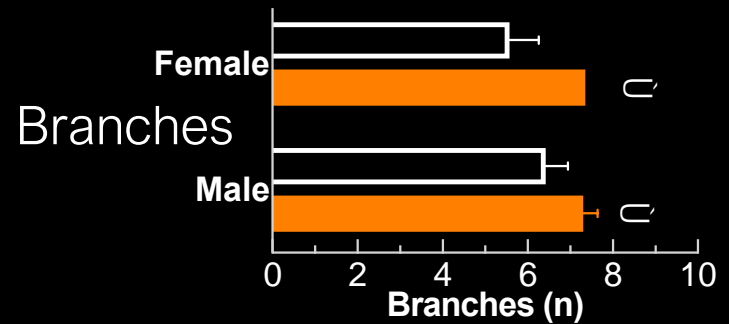
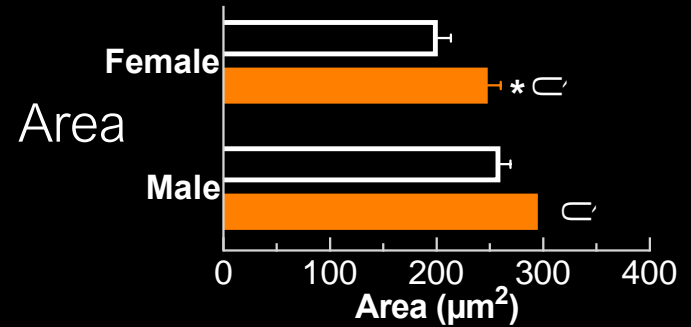
Male



Female



Increased microglia ramification





# Prenatal TLR7 Activation

## Summary

	Male	Female
Activity	↑ Increase*	↑ Increase*
Microglia	↓ Decrease	↓ Decrease
Microglia Morphology	↑ Increased ramification	↑ Increased ramification

# Prenatal TLR7 Activation

## Summary

	IMQ	Poly IC + LPS
Pup USVs	↓ Decrease	↑ Increase
Anxiety-like	↓ Decrease	↑ Increase
Repetitive	↑ Increase	↑ Increase
Social Interaction	↑↓ Fragmented	↓ Decrease
Activity	↑ Increase*	↓ Slight decrease
Microglia	↓ Decrease	↑ Increase

# Final thoughts

- Accumulating evidence for an immune involvement in ASD and other psychiatric disorders
- Different types of maternal immune activation may have divergent outcomes
- Largest hurdle is finding endpoints that translate from the lab to humans



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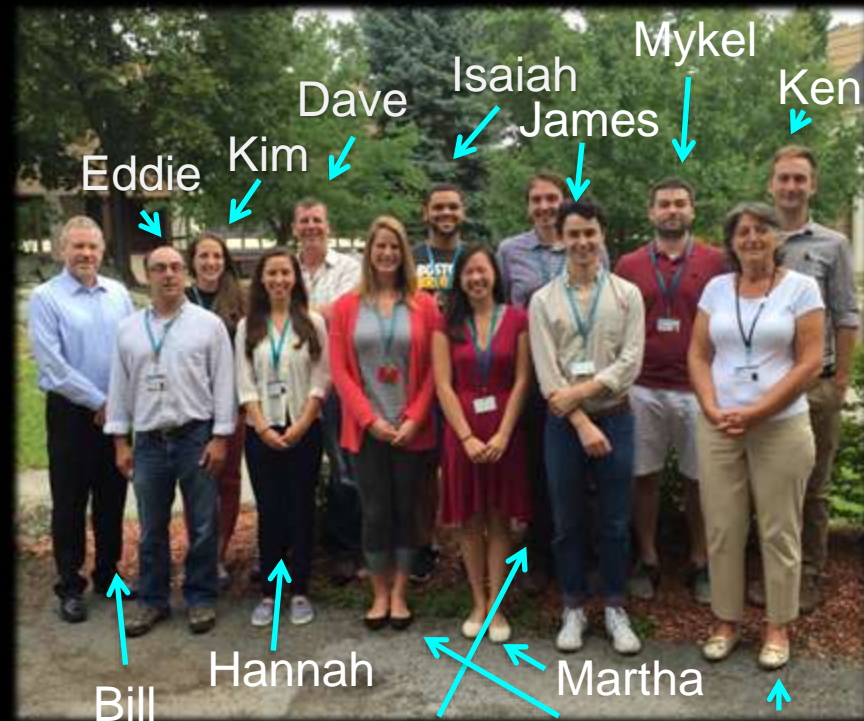
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# Final thoughts - Questions

- Accumulating evidence for an immune involvement in ASD and other psychiatric disorders
- Different types of maternal immune activation may have divergent outcomes
- Largest hurdle is finding endpoints that translate from the lab to humans